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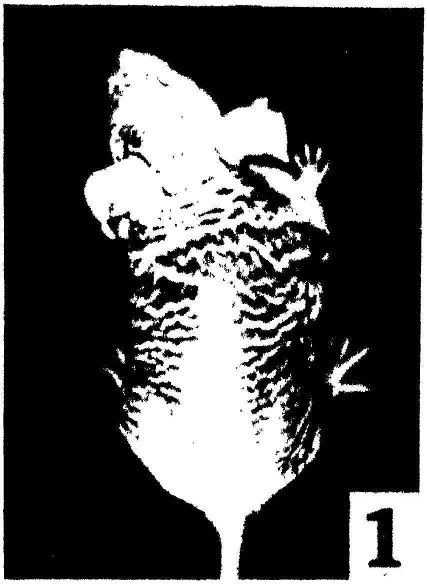
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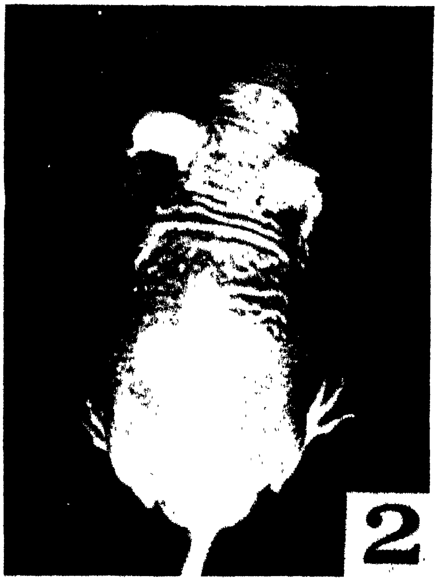
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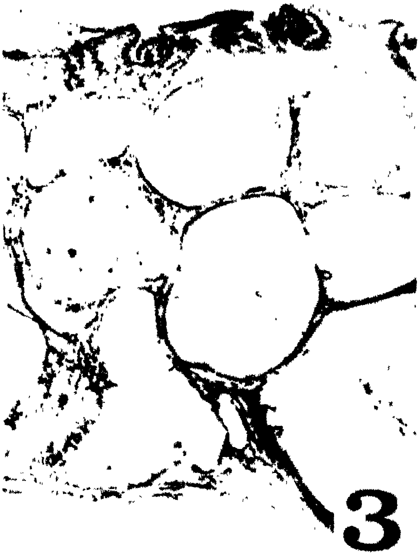
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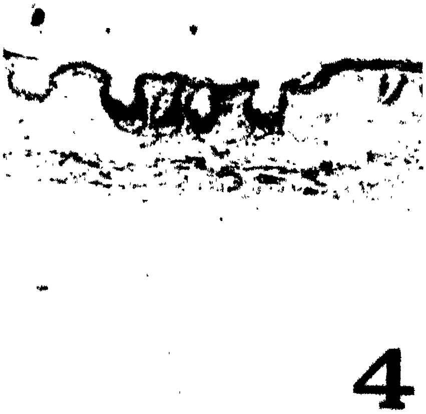
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THE WEIGERT-PAL METHOD FOR PARAFFIN-EMBEDDED MATERIAL

I. BULK STAINING AND STAINING OF PARAFFIN SECTIONS[1]

BY J. A. COLIN NICOL[2]

WITH THE ASSISTANCE OF FRANK PAUL[3]

Abstract

Three methods of staining myelin sheaths by the Weigert-Pal method in paraffin sections are compared. Hyomandibular nerves and spinal cord of the ratfish (*Hydrolagus colliei*) and tibial and peroneal nerves of the white rat were used. Fixation and mordanting comprised: 10% neutral formol, 48 hr.; 2.5% potassium bichromate at 37° C., 12 days; gliabeze, 2 days. The tissue was washed for 24 hr., and stained for 24 hr. either in bulk, or while in paraffin sections, or on the slide after coating with celloidin. Kulschitzky's haematoxylin, freshly prepared and artificially ripened, was used for staining. Differentiation was carried out by Pal's method. Bulk staining gave most satisfactory results, with the other methods as second and third alternatives, respectively. Treating the sections with safranin and dehydrating in dioxan was found to give a satisfactory counterstain.

Introduction

The Weigert method for staining myelin sheaths of nerve fibers involves treating the tissue with potassium bichromate, which acts as a mordant, then overstaining with haematoxylin to form a lake, and differentiating the sections. The bichromate is followed by a secondary mordant containing chrome and copper salts, which enhance the stain. Sections are usually cut in celloidin (1, 4). Also the method has been employed with paraffin embedding and, in common with other workers, we have obtained very good pictures of myelin sheaths in paraffin sections (8, *et al.*). One serious drawback to the paraffin method, however, is that sections frequently become detached from the slides during the prolonged period of immersion in the staining solutions, particularly if alkaline media are employed. This difficulty may be obviated by coating the sections with celloidin. We have found that better preparations may be obtained by these two methods: (*a*) staining the nerve in bulk after mordanting; (*b*) staining the sections while they are still in paraffin.

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[1] *Manuscript received September 3, 1948.*
Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C.

[2] *Assistant Professor of Zoology.*

[3] *Graduate Student.*

[The December issue of Section D (Can. J. Research, D, 26 :329-357. 1948.) was issued January 26, 1949.]

Bulk staining with alum haematoxylin is well known and the principle as we have employed it involves nothing radically new. Staining of material in paraffin has also been used by several workers. Baker (2) has shown that certain refractory materials may be softened by soaking the paraffin block of tissue in a glycerine–water–alcohol mixture that penetrates the surface tissue. Copeland (3) has obtained surface staining by placing the paraffin block in a solution of haematoxylin. Several workers have shown that sections in paraffin may be stained by floating the sections on the surface of the staining solution (5, 10, *et al.*) and Robb-Smith (7) has recommended this expedient for a silver impregnation method employing an alkaline solution that tends to lift sections from the slide. We have tried several variants and have found the following procedures satisfactory.

Materials and Methods

The material used comprised the hyomandibular nerve and spinal cord of the ratfish (*Hydrolagus colliei*) and the tibial and peroneal nerves of the white rat. Material was fixed for two days in 10% formol (4% formaldehyde), which was neutralized by the addition of powdered chalk. Subsequent procedure was as follows.

A. *Mordanting*

1. The nerves were placed in 2.5% potassium bichromate at 37° C. for 12 days.

2. They were transferred to a solution of gliabeze at room temperature for 48 hr. Gliabeze consists of chromium fluoride 2 gm., copper acetate 5 gm., glacial acetic acid 5 cc., and 100 cc. of distilled water.

3. Material was washed 24 hr. in running tap water.

B. *Staining of Sections*

The nerves were dehydrated in alcohols, cleared in cedarwood oil, embedded in paraffin wax (melting point about 56° C.), and sectioned at 15μ to 20μ.

Kulschitzky's acid haematoxylin, made up as follows, was used for staining: dissolve 1 gm. of haematoxylin in 10 cc. of 95% ethanol. Add 88 cc. of distilled water and 2 cc. of glacial acetic acid. Ripen for immediate use by the addition of 0.1 gm. of sodium iodate. Filter.

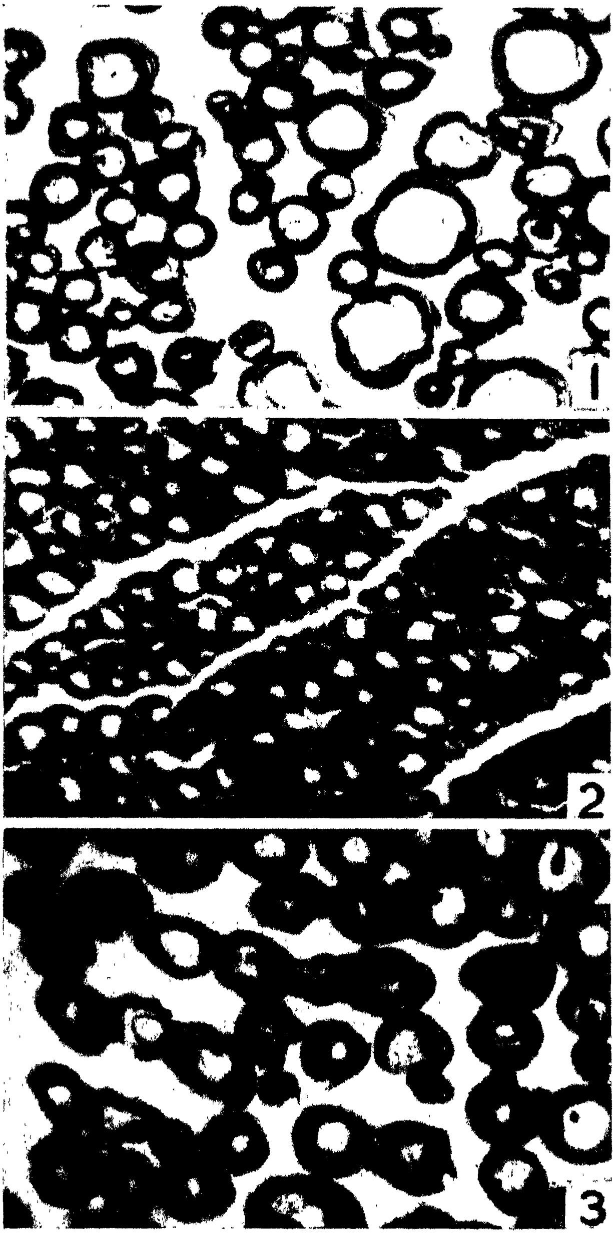
1. Sections were deparaffinized, coated with celloidin, hydrated, and incubated in the haematoxylin solution at 37° C. for 24 hr. They were then washed about 30 min. in running tap water and differentiated.

2. Sections, still in paraffin, were floated on the surface of the haematoxylin solution and placed in an incubator at 37° C. for 24 hr. They were removed then to a large volume of tap water, which washes off the stain, and mounted on slides. After drying, sections were deparaffinized, hydrated, and differentiated.

C. *Bulk Staining*

Mordanted nerves were placed in Kulschitzky's haematoxylin at 37° C. for 24 hr. and 48 hr. After washing for five hours in tap water they were

PLATE I



Photographs of transverse sections of nerve fibers stained by the Weigert-Pal method. Magnification ×560. FIG. 1. *Hyomandibular nerve of Hydrolagus colliei. Section stained while in paraffin. Fig. 2. Tibial nerve of rat. Nerve stained in bulk.* FIG. 3. *Hyomandibular nerve of Hydrolagus colliei. Section coated with celloidin and stained on the slide.*

embedded and sectioned as above. Sections were mounted, deparaffinized, hydrated, and differentiated.

D. *Differentiation*

This was done by Pal's method of alternate baths of potassium permanganate and oxalic acid – sodium sulphite solutions (4).

E. *Subsequent Treatment*

Sections were washed for one hour in running tap water and either mounted directly or counterstained with safranin (safranin rein, Grübler's). Safranin washes out very quickly in alcohols but may be retained by dehydrating the sections with dioxan, in which it is only slightly soluble (6, 9). Our procedure is as follows: stain 30 sec. in a 1% solution of aqueous safranin. Wash 5 min. in running tap water. Dehydrate in two changes of dioxan for 5 and 2 min. respectively. Clear in xylol and mount.

Results and Conclusions

Satisfactory preparations were obtained by all of the above methods (Figs. 1 to 3). Myelin sheaths appeared dark blue-black against a colorless background of connective tissue, or against a red background if the sections were counterstained. Without celloidin coating, however, most of the sections were lost from the slide. With a celloidin coating, and many sections on the slide, there is often a considerable variation in the degree of staining between different sections. This difficulty apparently arises through slight variations in the thickness of the celloidin film affecting diffusion times in the permanganate bath. With sections stained in paraffin some difficulty was encountered in obtaining perfectly flat sections owing to imbibition of water and swelling of the tissue. The procedure also is slightly more time-consuming than bulk staining. With this material bulk staining for 24 hr. gave uniformly good coloration of myelin sheaths. The latter method is recommended for peripheral nerves, with staining of sections in paraffin and staining of mounted sections as second and third alternatives. Small pieces of central nervous tissues may also be stained by the above methods.

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THE WEIGERT-PAL METHOD FOR PARAFFIN-EMBEDDED MATERIAL

II. DECALCIFICATION OF MATERIAL CONTAINING BONE[1]

By J. A. Colin Nicol[2]

With the Assistance of Frank Paul[3]

Abstract

Several methods were tried of decalcifying material to be stained by the Weigert-Pal method for myelin sheaths. Pieces of the tail of a teleost, *Porichthys notatus*, and peroneal and tibial nerves of the white rat were used. The following procedure was found to give good staining of myelin sheaths after treatment with acid. Fix tissue in 10% neutral formol. Transfer to 5% trichloroacetic acid for five days. Wash one hour, and mordant in potassium bichromate followed by gliabeze. Prepare paraffin sections, stain with Kulschitzky's haematoxylin, and differentiate by Pal's method.

Introduction

In using the Weigert-Pal method for the nervous system of small animals we have found it necessary to stain the nerves along with the contiguous tissue and to decalcify the bone. Having discovered no satisfactory technique in the literature we have tried several procedures, which are here described. Decalcification by trichloroacetic acid as reported in Bolles Lee § 601 (5) has been used. On empirical grounds the treatment of nervous tissue with acid should present no difficulty in carrying out a Weigert stain since Holmes (4) has reported that the addition of 5% acetic acid to the formol fixative preserves myelin sheaths for long periods (nine months). We have also tried the effects of adding calcium and cadmium to the various solutions, as recommended by Baker (1) for the preservation of lipides.

Materials and Methods

Two sources of tissue were used. (1) Blocks of the tail of the midshipman (*Porichthys notatus*), a teleost fish. These were 0.5 to 1 cm. cubes containing vertebral column, spinal cord, spinal nerves, haemal canal, and muscle. (2) Peroneal and tibial nerves of the white rat. These were used to provide an independent source of information concerning the action of the decalcifying agent on nerve fibers since the staining of their myelin sheaths by a routine method had already been determined. The fish tissue was fixed in 10% neutral formol for two months; the rat nerves were fixed for two days, in part in 10% neutral formol, in part in 10% neutral formol with the addition of 1 gm. of calcium chloride per 100 cc. Subsequent treatment consisted of

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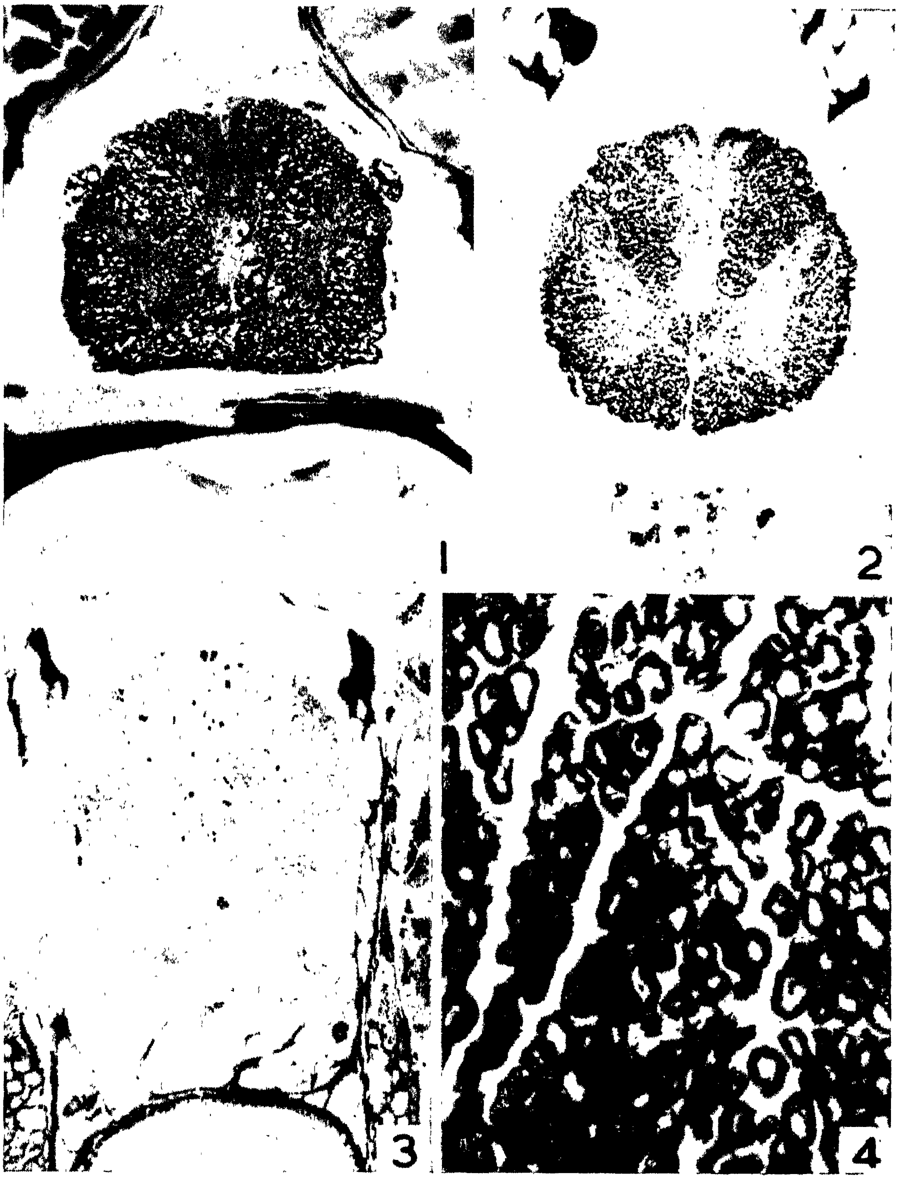
[1] *Manuscript received September 3, 1948.*

Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C.

[2] *Assistant Professor of Zoology.*

[3] *Graduate Student.*



Photographs of transverse sections of nervous tissue. Weigert-Pal stain.

FIGS. 1 to 3. *Spinal cord of Porichthys notatus. Magnification ×68.* FIG. 1. *Trichloroacetic acid followed by mordant. Counterstained with safranin.* FIG. 2. *Trichloroacetic acid—Ca-Cd followed by mordant.* FIG. 3. *Mordant followed by trichloroacetic acid.* FIG. 4. *Tibial nerve of white rat. Trichloroacetic acid followed by mordant. Magnification ×700.*

treating a portion of each material with the decalcifying agent, trichloroacetic acid, before mordanting; the remainder with trichloroacetic acid after mordanting.

Mordanting, staining, and differentiation were carried out in the manner described in the previous paper of this series.* The procedure was as follows.

A. *Decalcification Before Mordanting*

Tissues were transferred from the formol fixative to: (1) 5% trichloroacetic acid; (2) 5% trichloroacetic acid containing 1 gm. of calcium chloride per 100 cc.; (3) 5% trichloroacetic acid containing 1 gm. of calcium chloride and 1 gm. of cadmium chloride per 100 cc. for five days. They were then washed for one hour and mordanted. After paraffin embedding, sections were coated with celloidin, stained with Kulschitzky's haematoxylin, and differentiated by Pal's method.

B. *Decalcification After Mordanting*

Tissues were mordanted, washed, and transferred to the three solutions of trichloroacetic acid, described in Section A, for five days. They were then washed one hour and embedded in paraffin. Sections were coated with celloidin, stained with Kulschitzky's haematoxylin, and differentiated by the Pal method.

Some sections were counterstained with safranin by the procedure described in the previous paper.

Results and Conclusions

Material treated with trichloroacetic acid before mordanting gave good coloration of the myelin sheaths, which appeared dense blue-black. There was some background stain in the center of muscle fibers, and ossein and fatty cells were stained. Connective tissue, however, was unstained (Figs. 1 to 4). The method gives clear-cut demarcation of myelin sheaths in the nerve fibers. In material treated with trichloroacetic acid after mordanting the stain is weak and diffuse and washes out in the alcohols during dehydration. Weak acids have been used by several workers to remove chrome salts from sections that have been fixed in Helly's fluid or postchromed (2, 3) and it is probable that the trichloroacetic in this material has diminished or removed the chrome and copper mordant. No significantly better results were obtained by adding calcium and cadmium to the formalin and trichloroacetic acid. However, Pal's method of differentiation cannot be timed exactly from slide to slide and makes an exact comparison of staining intensity of doubtful value. For larger pieces of tissue requiring longer periods of decalcification it probably would be advantageous to add calcium and cadmium salts to reduce loss of lipides. We conclude that decalcification may be carried out successfully on tissue to be stained by the Weigert-Pal method. After preliminary fixation in 10% neutral formol, the tissue may be decalcified with 5% trichloroacetic acid, then mordanted, stained, and differentiated by the Pal method.

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* *Can. J. Research, D, 27 : 1-3. 1949.*

Acknowledgments

This work was carried out during an investigation of the autonomic nervous system of fish. We acknowledge with thanks financial assistance received from the National Research Council of Canada for this investigation.

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POPULATION PHENOMENA IN CERTAIN GAME FISHES OF LAKE SIMCOE, ONTARIO, AND SOME EFFECTS UPON ANGLING RETURNS. PART I. THE SMALLMOUTH BASS, *MICROPTERUS DOLOMIEU* LACÉPÈDE[1]

By James R. Westman[2] and Charlotte B. Westman

Abstract

The varying susceptibility and availability of game fish populations to angling methods is seldom approached by modern scientific procedure, despite the fact that angling returns are generally accepted as reliable indices of population densities. Lake Simcoe—Ontario's fourth largest lake—afforded an excellent opportunity to study a situation of long standing wherein a great abundance of smallmouth bass had long yielded generally poor angling returns.

Results from a scientific investigation, conducted mostly during the summer of 1940, were as follows:

(1) A somewhat radical angling method was developed which yields greatly increased and highly satisfactory returns from the bass population.

(2) The discovery that shifts in habitat—both diurnal and otherwise—by particular age groups of the smallmouth bass population had a significant effect upon angling returns.

(3) The discovery that the smallmouth bass population of the lake was heterogeneous in character, i.e. that bass from one section of the lake had a significantly more rapid rate of growth than bass from another section, and that this influenced the quality of catch.

(4) The conclusion that the smallmouth bass problem of Lake Simcoe cannot be solved by stocking; but rather by the dissemination of knowledge on improved methods of utilization.

Introduction

Contrary, perhaps, to popular conception, fishery problems are often concerned with situations of abundance and the need for discovering new techniques or methods that can more fully utilize a particular resource. Among the many types of such problems of "wasted abundance" may be mentioned the following: (1) unsatisfactory size composition of the population; (2) potential uses unknown or unappreciated; (3) danger to health through use; (4) satisfactory techniques, or methods, of processing unknown or unappreciated; (5) unsuitable regulations; (6) poor availability or susceptibility, or both, to employed methods of capture.

The last mentioned type of problem (poor availability, etc.) to which Lake Simcoe belongs, is apt to be difficult of solution for at least two reasons: first, because a situation of abundance may—owing to the inefficiency of the methods of capture being employed—masquerade under an appearance of scarcity; secondly, because the development of techniques, or methods, that are at once practical and feasible, may entail the prospect of considerable time and experimentation without any assurance of eventual success. With luck and a bit of persistence, however, the results of such efforts may be worthwhile and useful, as is indicated by the present research.

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[1] *Manuscript received October 9, 1948.*

[2] *Senior Aquatic Biologist (Marine), State of New York, Conservation Department.*

The Fishery Problem of Lake Simcoe

Lake Simcoe—Ontario's fourth largest lake—presents an interesting example of "scarcity midst abundance". It supports heavy populations of both lake trout and smallmouth bass, yet the former are caught in quantity (by angling) only during the spring of the year, while the latter are seldom taken consistently and in number except in the northern section of the lake by relatively few anglers (including professionally guided parties). This condition, which exists today, has endured during the memory of the senior author, which extends back prior to the year 1922.

The abundance of smallmouth bass in Lake Simcoe cannot very well be questioned, because in the Kempenfeldt Bay area, where angling returns for this species are possibly the poorest of any region of the lake, the water is so clear during the early part of the summer that fish can be observed at depths up to 10 ft. or more; and the number of smallmouth bass that can be seen by the careful observer is truly remarkable. On calm, sunny days, for instance, literally hundreds of legal-sized bass can sometimes be observed during an hour's slow trip in a power boat, distributed singly or in groups of two or more, over the extensive areas of sandy and stony littoral. An additional opportunity to witness the density of these fish in the Kempenfeldt Bay area is afforded during the flights of the mayfly, *Hexagenia* sp., usually during the early part of July. On these occasions, one can make a leisurely trip by power boat along the southern shore of Kempenfeldt Bay, from Lovers' Creek to Big Bay Point (see Fig. 2), and seldom be out of sight of smallmouth bass feeding upon these mayflies.

As previously indicated, the smallmouth bass in Lake Simcoe have a very poor susceptibility to conventional methods of angling, except within certain localities at particular times of year. And even in these areas, experience and special knowledge, such as that possessed by a few professional guides, have been necessary for consistent results. During 14 summers prior to 1939, for example, the senior author, aware of an abundance of bass in the Kempenfeldt Bay area, experimented with a wide variety of baits and commercial lures, but could never catch smallmouth bass in any number or with any degree of consistency. The best returns resulted from trolling during very cloudy days or late evening, but even these rewards were few and far between.

It was perhaps inevitable that under such circumstances Lake Simcoe should have early attained the reputation of being a "poor bass lake", and that the generally poor angling returns should have been widely interpreted as conclusive evidence of population scarcity. Rawson (7), for example, concurred in this conclusion, although he correctly reported the lake trout problem as being one of poor availability to angling methods during the summer months. (It is interesting to note that while Rawson (7) recommended the stocking of smallmouth bass and rainbow trout as solutions to these problems, both these measures appear highly undesirable in the light of the present research.)

Since the fishery problem of Lake Simcoe was so obviously one of inadequate methods of utilization, the writers decided, in 1939, to make intensive efforts toward developing effective angling methods for taking the smallmouth bass and lake trout; and, if successful, to conduct careful studies of these populations during the season of 1940; to be followed in subsequent years with checks upon results from the continued use of such angling methods or any modifications of same that might be developed.

Description of the Lake

Lake Simcoe is situated in gently rolling land of glacial moraine origin, about 40 miles north of the city of Toronto. The general shape and large size of the lake may best be understood by examining the map (Fig. 1). The surface area exceeds 275 square miles and the shoreline is more than 140 miles in length, including islands. The position of the lake has made it a connecting link in the Trent Valley system of waterways, which provides a navigation route from Lake Ontario to Georgian Bay. The elevation of Lake Simcoe is 720 ft. and its natural drainage is into Georgian Bay by way of Lake Couchiching and the Severn River. The connection between Lakes Couchiching and Simcoe is a narrows through which flows a considerable volume of water.

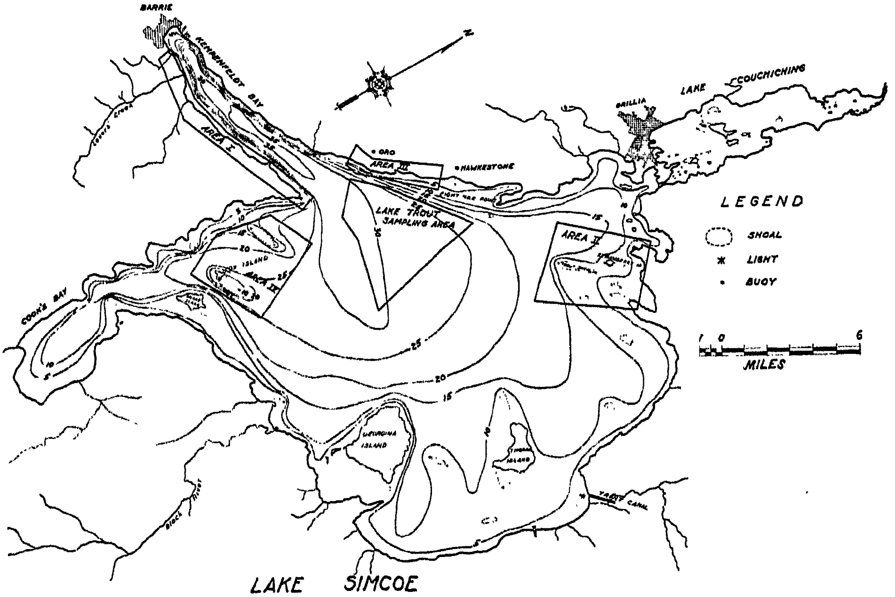

FIG. 1. *Map of Lake Simcoe, Ontario. The depth contours, in meters, are after Rawson (7) but have been slightly modified.*

The water of the lake may be described as blue-green and clear, being similar in appearance to the waters of New York's finger lakes. During late July, August, and early September, the clarity of the water in Kempenfeldt

Bay (as measured by distances of bottom visibility) appears to be nearly twice that of the water in the northern section of the lake. The bathetic character of Lake Simcoe is shown by the map (Fig. 1).

Lake Simcoe may be considered as intermediate in type between the eutrophic and oligotrophic categories. Rawson (7) has termed it "an eutrophic lake with an oligohumus bottom". There is a moderate stratification of water during the summer months when the bottom temperatures in the deeper sections may range from 9° to 14° C. and when the dissolved oxygen content near the mud, according to Rawson (7) may be as low as 2.0 p.p.m.

Of particular interest is the character of the shoreline and the presence of extremely large shoals, some of which are more than a mile in length. Rawson (7) has grouped the various types of shoreline as follows: 54% stony; 33% sand; 13% vegetation. The shoals are mostly gravel to boulder in character, except when forming shelves along the shoreline, in which case they may be sandy with patches of weed beds. The gravel, rubble, and boulders are chiefly limestone and have been worn smooth by erosion. Boulders are frequently large-sized, sometimes exceeding 8 ft. in diameter.

Procedure

Briefly outlined, the experimental procedures may be listed as follows:

1. The development of an effective angling gear and method.
2. The establishment of sampling areas in different parts of the lake, and the procurement and study of samples of the bass population from these areas.
3. The consistent use of the developed angling method throughout the summer of 1940 in one of the sampling areas.
4. The procurement of miscellaneous data on such phenomena as water temperatures, weather conditions, parasitism, spawning sites, etc.

The field investigations were made during September, 1939; from June 13 to Oct. 15, 1940; and again during a brief period in July, 1946. In addition, the developed angling method or modifications of same, or both, were used by other persons during the summers following 1940, and comment was received on the general results obtained from these angling efforts.

1. THE ANGLING METHOD

A very effective angling method for taking Lake Simcoe bass was developed during September, 1939, after a series of discouraging experiments. It consisted essentially of trolling a bucktail streamer fly (of home design) and spinner in the wash of a power boat at a speed of approximately two to three miles per hour. The usual trolling distance of the lure was from 35 to 60 ft. astern, and it was early discovered that the bucktail fly should never be permitted to spin if good results were to be obtained.

Two bucktail flies, each of a different color pattern, were found to be equally effective. One of these flies may be described as follows: It possessed a lemon-yellow, chenille body with red tail; a head of black thread tightly wound and varnished; and a layer of yellow bucktail hair covered dorsally by a layer of red bucktail. It was tied on a No. 5 long-shanked hook and differed importantly from commercial lures of its type by being streamlined, i.e. the hair was almost horizontal to the body when the fly was dry, and completely horizontal when being trolled.

The spinner was a No. 2 hammered brass, and a 31-in. leader of 8 lb. test synthetic gut was tied directly to the spinner shank, with the other end of the leader fastened to a small swivel. A No. 6 or 7 clamp sinker was fixed on the leader directly below the swivel. The line was 12.5 lb. test nylon, and it was discovered that if a coarser line was employed, a slightly heavier sinker was preferable.

The boat chiefly used for the smallmouth bass angling during 1940 was a 15 ft. yacht dinghy, powered by a 5 h.p. outboard motor equipped with a slow speed adjustment. This motor had an underwater exhaust, and it was surprising to note that large bass were sometimes taken when the lure was within 10 ft. of this exhaust and the revolving propeller. It was also interesting to discover that the casting of the same lure and rig noted above was singularly ineffective, even in spots where a number of fish would be taken a few moments later by the trolling method. This experiment was tried again and again with similar results, and it suggests that the wash of the propellor and boat were important factors in the efficiency of the method. This, of course, is not incredible when the apparent importance of "boat wash" to certain salt water trolling methods is considered. A small launch was also occasionally employed during 1940, and this craft seemed to be just as efficient as the outboard.

Experiments by other persons after the summer of 1940 revealed that small plugs of the diving type could also be trolled with considerable success, and this modification is now preferred by some in the belief that it is selective for the larger bass. It also yields good returns when a paddled canoe is used.

During the 1940 experiments, all trolling was done within the last five and one-half hours of daylight. This meant that at the beginning of the summer, a day's angling did not cease until approximately 9:10 P.M. Eastern Standard Time, when daylight had diminished to a point where reading ordinary newsprint was extremely difficult. The number of lines trolled simultaneously was usually two (by two persons) although as many as four lines (by four persons) were trolled on occasion. Throughout the entire period of study, all angling methods, procedures, and operations were made to conform with the official regulations that governed the taking of smallmouth bass in these waters.

2 AND 3. AREAS AND SAMPLING PROCEDURES

Four reference areas were set up for population sampling (Fig. 1). Of these, Area I was subdivided into six zones (Fig. 2). This particular area differs from the others by possessing a shore shelf which extends, almost uninterrupted, along its entire length. This shelf varies in composition from sand, with weed patches, to boulders, and has a maximum depth of approximately 15 ft. The outer edge of the shelf, where the lake deepens suddenly, has intermittent weed beds of *Potamogeton* sp. It was possible to troll this shelf to within a few feet of the shore, where the water is approximately 2 ft. deep.

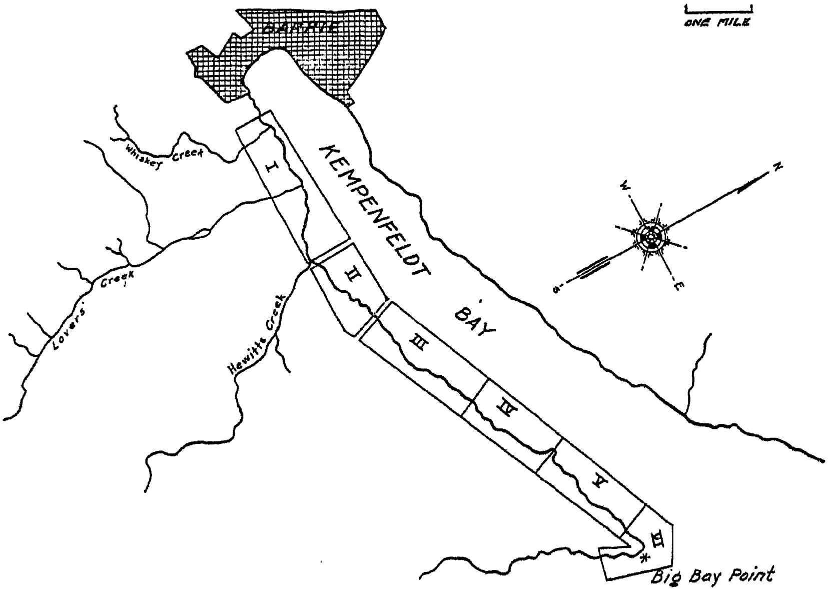

FIG. 2. *Map of Kempenfeldt Bay showing the subdivision of Area I into six zones.*

Area III has a comparatively narrow shore shelf of rubble and boulders with an occasional sandy region. No weed beds were noted in this area.

Areas II and IV possess large shoals whose depths generally range from about 18 in. to 20 ft. The shoals in the latter area, and some in the former area, are much exposed to wave action, as can be seen from Fig. 1.

Area I was fished intensively during July, August, and part of September, 1940, when the trolling method was used exclusively. Owing to weather conditions, it was not practical to sample this area every day during the period in question, but a total of 30 fishing days were accounted for during July and August. Area II was sampled on three days during August and on two days in September, when both trolling and bait fishing methods were employed. The two remaining areas were fished from time to time during the summer by the trolling method, more in an attempt to determine angling conditions than to obtain samples for comparative study.

Data on the fish from the various sampling areas were obtained as follows. The lengths of the specimens were measured from snout to center of tail to the nearest half-centimeter. Scale samples were taken from two regions of the body: from a small area on the side just posterior to the tip of the relaxed pectoral fin (approximately 16 scales), and from the side just above the lateral line and ventral to the soft dorsal fin. Scales were taken from the second region merely to ensure a reserve sample in the event that the scales from the first region proved to be regenerated. Weights of sublegal-sized fish were obtained directly after scale sampling and measuring, whereupon these small specimens were returned to the water. Weights of those larger fish that were not returned to the water were always obtained within five hours from the time the specimens were taken; and those smallmouth that were killed and retained were always kept in a moist condition until weighed. All weights were obtained to the nearest half-ounce by means of a postal spring scale that had a capacity of four pounds. Stomachs were removed from the retained specimens, immediately after the weights were recorded, and were then wrapped in cheese cloth, labelled, and placed in 10% formalin for future study.

A total of 105 of those bass that were caught and returned to the water in Area I during the period July 4 to Aug. 19 were marked by fin clipping. This experiment will not be reported upon fully because of the small number of returns. However, those returns that were obtained tended to demonstrate that fins clipped in the manner employed for this experiment would not regenerate.

4. Miscellaneous Observations

Bottom and surface temperatures were taken in the several zones of Area I from time to time throughout the season of 1940. Temperatures were also taken in Zone I during a two week period directly preceding the opening of the angling season.

Results

Results of Angling in Area I, and Analysis of Catch

It has been previously indicated that the angling effort expended in Area I was for three main purposes: first, to demonstrate an angling method that would yield excellent results as viewed by fishermen; secondly, to reveal changes (if any) in the susceptibility or availability of the bass population to this angling procedure; and thirdly, to obtain samples of the bass population in all the zones throughout the period in question. The angling effort, therefore, was not devoted toward obtaining the maximum number of fish per effort from the best sections of the area. It would have been a simple matter, for instance, to have concentrated any day's angling to the zone or place where the most fish had been caught previous to that day. However, all the zones received attention regardless of the angling results obtained. It should be re-emphasized, moreover, that Kempenfeldt Bay, where Area I was situated, has possibly the poorest reputation for bass angling of any region in the entire lake. Consequently, the results of the angling effort in this area should be regarded in the light of several mitigating factors.

During July and August, a total of 197 bass were taken in Area I. The 15 angling days in July accounted for 117 of these fish while an equal number of days of angling in August produced only 80. Six fishing days in September accounted for only four bass, and two days of angling in October failed to produce any catch.

The lengths of the fish taken during July and August (Table I) have been plotted by their percentage frequency of occurrence in Fig. 3. The age composition of this catch is also revealed by Fig. 3. A study of these graphs immediately suggests that a change in susceptibility or availability of the larger fish (those of four years of age and over) took place during the period in question. Only 20 fish over the legal size limit, or approximately 25% of the August catch, were taken during that month; while 70 legal-sized bass, or approximately 60%, were taken during the July period. These data, however, do not reveal the entire picture because more than half of the 20 legal fish that were taken during August were caught in much deeper water and nearly always in weed beds of *Potamogeton* sp. When only those fish that were taken from similar habitat are considered, there is less than one chance in one hundred (based upon Chi square) that the difference in catch of these larger fish between July and August was due to chance. This drop in catch was

TABLE I

Lengths of smallmouth bass taken in Area I during July and August, 1940

Length, $\frac{\text{cm.}}{2}$	No. fish, July	No. fish, August	Length, $\frac{\text{cm.}}{2}$	No. fish, July	No. fish, August
26	0	0	55	2	1
27	1	0	56	5	0
28	0	0	57	5	2
29	1	0	58	4	1
30	0	1	59	2	2
31	3	0	60	3	0
32	1	1	61	3	0
33	0	0	62	1	1
34	1	3	63	4	1
35	0	0	64	3	1
36	0	3	65	1	0
37	1	5	66	0	0
38	3	1	67	0	0
39	4	2	68	2	0
40	7	2	69	3	0
41	4	3	70	4	0
42	1	8	71	2	1
43	5	6	72	1	1
44	4	5	73	3	2
45	2	11	74	3	2
46	1	4	75	1	0
47	5	1	76	1	0
48	1	3	77	3	0
49	1	0	78	1	0
50	1	1	79	0	0
51	3	1	80	0	0
52	1	2	81	1	0
53	5	1	82	0	0
54	3	1			

sudden and took place during the first few days of August, when it was also noted that there was a rapid decrease in the number of bass that could be seen from the boat. In fact, a legal-sized fish was seldom seen in the "July" area after Aug. 3, and, as previously mentioned, the majority of those fish that were caught in Area I after this date were taken from the deeper waters and in weed beds, particularly along the outer margins of the shelf. Finally, the remaining legal-sized fish that were caught during August, in this Area, were, without exception, taken within the last hour of daylight.

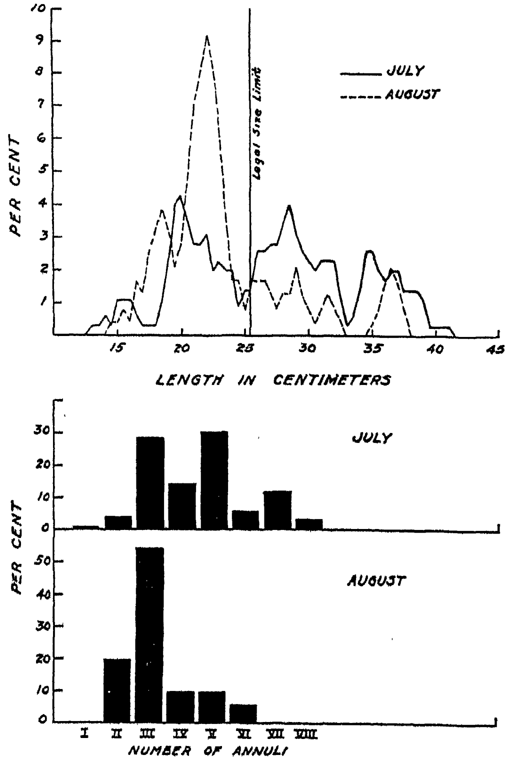

FIG. 3. *(Upper) Lengths of smallmouth bass taken from Area I during July and August, 1940, plotted by their percentage frequency of occurrence. These data have been smoothed once by a moving average of threes.*

(Lower) Age composition of bass taken from Area I during July and August, 1940.

The fact that the larger bass were most frequently taken in weed beds during August is of interest, because the adults of this species are often believed not to frequent such a habitat, when rocky areas are available. For example, Hubbs and Bailey (6) have stated that adult smallmouth "are rarely taken near beds of submerged or floating plants". Yet the instance noted here is not at all exceptional for Lake Simcoe; and in Lake Couchiching, in particular, the presence of adult smallmouth bass in weed beds is a common occurrence, especially during the months of August and September. In Area II, also, bass are frequently taken from beds of *Potamogeton* sp., and are sometimes purposely fished for in this type of habitat by professional guides.

The one-year-old age class of bass (average length undetermined) and most of the two-year-old fish (average length 30.8 half-centimeters in July and 36.5 ± .83 for August) were taken in regions of large boulders, docks, and sunken cribs. The depth of water in these instances varied considerably: from about 3 to 20 ft. The three-year-old fish (average length 41.2 ± .48 half-centimeters for July and 43.5 ± .38 for August) were usually taken in quiet, shallow water and very close to the shoreline. Hence there appeared to be several types of habitat occupied by smallmouth bass in Area I, with the particular habitat selected usually depending upon the age of the fish, the time of year, and the time of day. The presence of legal-sized smallmouth on the shelf region of Area I during mornings and afternoons, however, is not necessarily limited to the early part of summer. During September of 1939, for example, excellent catches from the shore shelf were made during daytimes, and similar returns were obtained during the years following 1940. Further, it has been noted that the relative "productiveness" of the several zones may change from one year to another.

The fact that the fish of the older age classes changed their habitat at approximate midsummer during 1940, leads to the consideration of possible causes for this phenomenon. Since all the legal-sized fish examined were adults, it was first suspected that spawning activity was concerned in the habitat change. It is true that the fish taken during July were caught in spawning regions. This was revealed by the presence of nesting sites, particularly in Zone III, which were generally located at depths of from 6 to 10 ft. However, only two of these nests were observed to be possibly occupied by adult fish after July 1.

Whether or not male bass were guarding young during July 1940 is a matter of question; but the fact that adult females were also present in the region during this time is shown by the records. Of the 49 sexed fish taken during July, for instance, 27 were males and 22 were females. Based on Chi square, the probability is approximately one to one that this sample is a fifty-fifty distribution of males and females. There was also no significant difference between the number of males and females taken during the first 16 days of July, when the sexed fish included 18 males and 13 females, nor during the last 15 days of July when nine males and nine females were examined. The change in habitat by the adult fish did not alter this sex distribution of the catch, since the August sample included 10 males and nine females.

These data would indicate that the parental care of nest or young, if present at the time, did not influence the sex distribution of the catch. Supplementing this observation is the fact that the type and action of the lures used was very influential in determining angling results for both the adult male and female fish. It seems probable, therefore, that the striking of the lure was not a protective response on the part of guarding male bass.

The cause for the decrease in catch of sublegal-sized bass during September seemed to be one of change in susceptibility. These fish appeared not to have changed their habitat during this time, yet catches by the trolling method were very low.

Growth Rates in Area I

The examination of the scales from fish taken in Area I indicated that the 1940 annulus was laid down during the late spring or early summer. Scales from most of the fish taken during the first two weeks of July did not have a clearly defined annulus at the periphery. The 1940 annulus, however, had become well defined by the fourth week of July. In the reading of the scales, this 1940 annulus was always included whether or not it was clearly defined on specimens taken during the early weeks of the season.

The age–length relationships of bass taken in Area I during July and August have been plotted in Fig. 4 by the method employed by Westman and Fahy (10) and Westman and Gilbert (11). The growth rates in Fig. 4 make no distinction between male and female fish, and, owing to the fact that the rates are used comparatively, it is desirable to know whether or not there are significant differences of growth rate between the sexes of these fish.

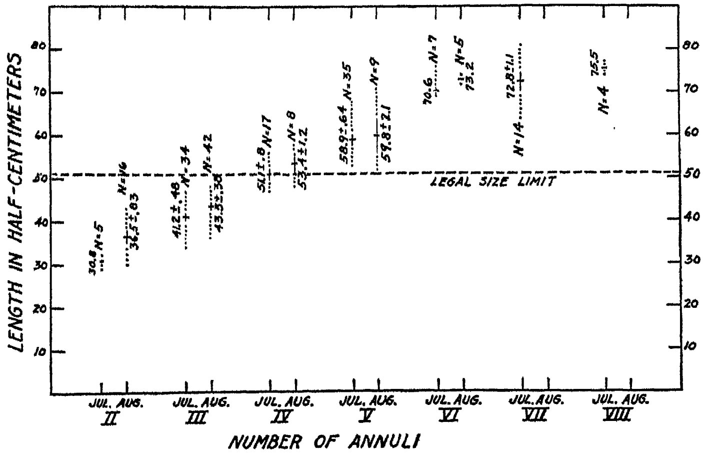

Fig. 4. *Age–length relationships of smallmouth bass taken from Area I during July and August, 1940. The solid line represents twice the standard error of the mean in both the plus and minus directions. The broken line represents the limits of variation found in the sample. The means, with their standard errors, and the numbers of specimens are also given numerically.*

Comparisons in the growth rate of the male and female bass from Lake Simcoe were, by necessity, limited to the five-, six-, and seven-year-old fish. Accordingly, the lengths of male and female bass of three age classes, taken from Area I during the period from July 4 to Aug. 16 (Table II), were transformed into three-place logarithms and compared by means of an analysis of variance. The results are as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	35	0.093615	
Age	2	0.071961	0.035980
Sex	1	0.000221	0.000221
Age–sex interaction	2	0.004927	0.002463*
Within subclasses	30	0.016506	0.000550

TABLE II

LENGTHS, IN HALF-CENTIMETERS, OF MALE AND FEMALE SMALLMOUTH BASS OF THREE AGE CLASSES TAKEN IN AREA I DURING THE PERIOD JULY 4 TO AUG. 16, 1940. THE DATA HAVE BEEN MADE PROPORTIONATE BY RANDOM SUBSAMPLING

No. of annuli					
V		VI		VII	
Male	Female	Male	Female	Male	Female
56	54	69	70	71	81
60	58	70	73	70	77
64	61	69	74	72	74
58	61	72		64	
62	57	74		68	
57	52	73		73	
58					
63					
59					
51					
64					
59					

No significant difference between the growth rate of the males and females is indicated by the foregoing analysis. There is, however, a significant age–sex interaction which suggests that differences may exist at particular times in life.

A comparison of 13 male and 19 female bass of the five-year-old class taken during the period from July 4 to Aug. 16 (Table III), gives an F value of zero, with the difference between the means of the non-transformed data being only 0.03 half-centimeters.

In dealing with the growth rates of male and female bass from Perch Lake, Ont., Tester (9) states: "The results, given in table 5, show that, on the average, female bass of Perch Lake grew at a slightly slower rate than the male bass." This statement has been mentioned by Greeley (2) and Bennett (1). A study of Tester's data, however, reveals the fact that his conclusions rest upon single averages, with normal variations due to chance not accounted for. This fact, particularly when the extent of variability in bass of the younger size groups is considered, apparently makes any significant differences of growth rate between the sexes yet to be demonstrated.

TABLE III

LENGTHS, IN HALF-CENTIMETERS, OF FIVE-YEAR-OLD MALE AND FEMALE SMALLMOUTH BASS TAKEN IN AREA I DURING THE PERIOD JULY 4 TO AUG. 16, 1940

No. of annuli			
V			
Male	Female	Male	Female
56	61	51	53
60	54	64	58
64	57	59	61
58	60		58
62	58		57
57	57		57
58	63		71
63	61		52
59	65		62
59	61		

Further attempts toward determining whether or not there are significant differences in growth rate between male and female bass during the early years of life were made as follows: From the published data of Tester (9), Greeley (2), Greeley (3), Greeley (4), Greeley (5), and Bennett (1), the mean lengths of male and female bass from various lakes were compiled, combined, and compared. The mean lengths of two-, three-, and four-year-old fish from seven lakes (see Table IV) were first taken, transformed to logarithms and analyzed. The results of the analysis of the variance are as follows:

TABLE IV

MEAN LENGTHS, IN MILLIMETERS, OF TWO-, THREE-, AND FOUR-YEAR-OLD MALE AND FEMALE SMALLMOUTH BASS FROM SEVEN LAKES, AS COMPILED FROM THE PUBLISHED DATA OF TESTER (9); GREELEY (2, 3, AND 5); AND BENNETT (1)

	No. of annuli					
	II		III		IV	
	Male	Female	Male	Female	Male	Female
Tester (9)						
Perch Lake	146	137	173	169	212	201
Bennett (1)						
Nebish Lake	179	177	213	226	270	239
Pallette Lake	176	173	229	196	249	235
Greeley (2)						
Raquette Lake	146	168	208	187	223	243
Duck Lake	166	156	192	189	216	212
Greeley (3)						
Gilboa Reservoir	182	184	247	206	273	249
Greeley (5)						
Kensico Reservoir	151	169	248	235	281	281

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	41	0.283413	
Age	2	0.193280	0.096640
Sex	1	0.001723	0.001723
Situation (time, place, and method)	6	0.064078	0.010680
Error	32	0.024332	0.000760

No significant difference in growth rate between males and females having been shown by the above, the mean lengths of two-, and three-year-old males and females from 12 lakes (Table V) were then compared in similar fashion:

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	47	0.345547	
Age	1	0.091700	0.091700
Sex	1	0.000075	0.000075
Situation	11	0.213691	0.019426
Error	34	0.040081	0.001670

Here again no significant difference is shown to exist between the growth rates of the male and female bass.

TABLE V

Mean lengths, in millimeters, of two- and three-year-old male and female smallmouth bass from 12 lakes, as compiled from the published data of Tester (9); Greeley (2, 3, 4, and 5); and Bennett (1)

No. of annuli

	II		III	
	Male	Female	Male	Female
Tester (9)				
Perch Lake	146	137	173	169
Bennett (1)				
Nebish Lake	179	177	213	226
Pallette Lake	176	173	229	196
Muskellunge Lake	187	185	229	241
Greeley (2)				
Raquette Lake	146	168	208	187
Duck Lake	166	156	192	189
Simon Pond	224	256	225	280
Tupper Lake	215	207	265	286
Forked Lake	133	128	159	171
Greeley (3)				
Gilboa Reservoir	182	184	247	206
Greeley (4)				
Lake Huntington	231	220	221	241
Greeley (5)				
Kensico Reservoir	151	169	248	235

From all the foregoing analyses, it seems evident that if differences in growth rate between the sexes do exist, then such differences are very slight and are perhaps confined to the older age classes. For this reason there seems to be no necessity for considering the growth rates of the male and female bass from Lake Simcoe in separate categories.

In order to determine whether significant differences of growth rate existed between bass from different regions of Area I, a comparison of the growth rates of fish from Zones I and II, taken during the period from July 24 to Aug. 20, was made with the fish from Zones V and VI taken during the same period of time. Accordingly, the lengths of the two groups of fish (Table VI) were transformed into three-place logarithms and the significance of difference determined by means of an analysis of variance. The results are as follows:

TABLE VI

LENGTHS, IN HALF-CENTIMETERS, OF TWO-, THREE-, FOUR-, FIVE-, AND SIX-YEAR-OLD SMALL-MOUTH BASS TAKEN FROM ZONES I AND II, AND FROM ZONES V AND VI, DURING THE PERIOD JULY 24 TO AUG. 20, 1940. THE DATA HAVE BEEN MADE PROPORTIONATE BY RANDOM SUBSAMPLING

No. of annuli

II		III		IV		V		VI	
Zone I & II	Zone V & VI	Zone I & II	Zone V & VI	Zone I & II	Zone V & VI	Zone I & II	Zone V & VI	Zone I & II	Zone V & VI
37	31	42	48	55	54	57	58	73	69
36	30	43	42	58	50	51	57	74	73
34	32	44	44			71	59		
		45	46			58	62		
		38	44						
		39	41						
		42	41						
		45	45						

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	37	0.425064	
Age	4	0.394615	
Place	1	0.000295	0.000295
Age–place interaction	4	0.006797	0.001699
Within subclasses	28	0.023357	0.000834

No significance of difference between the growth rates of the two groups of fish is demonstrated by the above and, consequently, when the rate of growth is used as the criterion, no evidences of population heterogeneity in Area I were found.

RESULTS OF ANGLING IN AREA II, AND ANALYSIS OF CATCH

During 1940, angling in Area II was done on Aug. 21, 23, 24, and on Sept. 16 and 22. The use of the trolling method was confined to Aug. 24 and Sept. 16 and 22, when its success was limited to the last hour of daylight. On the first two trolling days, a total of 11 bass were caught—all legal-sized fish which ranged in weight from 12 oz. to 3 lb., 3 oz., and which had an aggregate weight of 21.5 lb. The trolling on Sept. 22 yielded no fish, and, according to the professional guides, this was due to the usual fall "disappearance" of bass from the Area II shoals which "had taken place a few days previously". Approximately four hours of bait fishing with two lines on Aug. 21, and another four hours with five lines on Aug. 23, yielded a total of 39 fish, 20 of which were legal-sized and ranging up to two-and-one-half pounds in weight. It seems probable that a greater experience and familiarity with Area II would have yielded even more successful angling results than those that were obtained. The trolling method had not been employed by us in this area previous to the sampling days.

The catch from Area II was made up of fish from two-plus to eight-plus years of age, the eight-year class consisting of four bass that were 80 or more half-centimeters in length and that averaged 3 lb. in weight. The length–age relationships of the fish taken in Area II during August and September (Table VII) have been plotted in Fig. 5.

TABLE VII

LENGTHS, IN HALF-CENTIMETERS, OF SMALLMOUTH BASS TAKEN IN AREA II ON AUG. 21, 23, AND 24; AND ON SEPT. 16, 1940

No. of annuli

II*	III*	IV*	V*	VI*	VII*	VIII**
42	51	56	66	67	79	80
43	48	53	57	76	81	83
41	44	57	62	73		84
35	43	57	68	72		88
40	45	62	65			
38	44	66	59			
39	53					
36	56					
39	57					
34	51					
41	53					
	51					
	42					
	45					
	49					

* *August specimens.*

** *September specimens.*

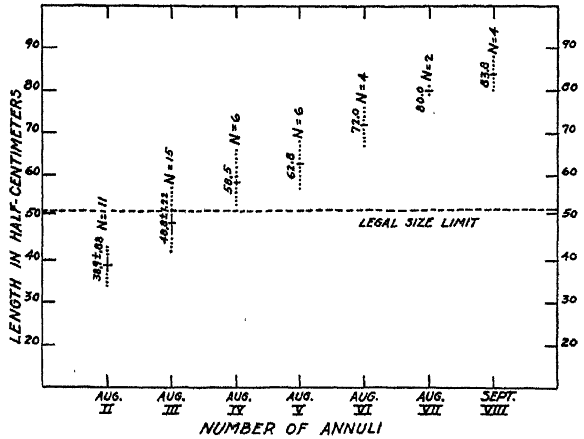

FIG. 5. *Age–length relationships of smallmouth bass taken from Area II. (For explanation of figure, see Fig. 4.)*

RESULTS OF ANGLING IN OTHER AREAS

Area III was trolled for approximately three hours on July 10, for one hour on Aug. 9, and for a half hour on Aug. 21. A total of 28 bass were taken on the three sampling days, 10 of these fish being of legal size. The July 19 catch contained four females and one male; and the Aug. 21 catch, one female and one male. A considerable number of very large bass were seen in this area, although only one was hooked.

Area IV was trolled twice during August, with a total fishing effort of approximately two hours. Thirteen bass were taken, five of which were over the legal size limit. This particular area was not fished during the last two hours of daylight.

A COMPARISON OF THE GROWTH RATES OF BASS FROM AREAS I AND II

A comparison of Figs. 4 and 5 immediately suggests that the bass in Area II have a considerably faster growth rate than the fish in Area I. The legal size limit of 10 in., for example, cuts through the length range of the August three-year-old fish in Area II, while in Area I it falls above the range of the three-year-olds, sampled during the same month, but crosses the four- and five-year-old length ranges. That a significant difference in growth rates did exist between the bass of the two areas was demonstrated by testing the significance of difference between the lengths of the 15 three-year-old bass taken in Area II, during August, with the 19 fish of the same year-class taken in Area I during the last two weeks of the same month. In testing the significance of difference, the lengths of the two groups of fish (Table VIII) were first transformed into three-place logarithms and then compared by means of an analysis of variance. The results are as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	33	0.052468	
Area	1	0.016711	0.016711**
Error	32	0.035757	0.001117

Since the probability is more than a hundred to one that the difference between the two groups is not due to chance, the question is now raised: "Have significant differences in the size of the two groups existed during their previous years of life?"

TABLE VIII

LENGTHS, IN HALF-CENTIMETERS, OF THE 15 THREE-YEAR-OLD SMALLMOUTH BASS FROM AREA II AND THE 19 THREE-YEAR-OLD BASS FROM AREA I, TAKEN DURING THE LAST TWO WEEKS OF AUGUST, 1940

Area I	Area II	Area I	Area II
45	51	45	53
45	48	45	51
48	44	38	42
43	43	46	45
45	45	42	49
45	44	44	
43	53	46	
42	56	41	
44	57	41	
46	51		

TABLE IX

DISTANCE READINGS OF ANNULI FROM THE SCALE FOCI FOR THREE-YEAR-OLD SMALLMOUTH BASS TAKEN FROM AREAS I AND II DURING THE LAST TWO WEEKS OF AUGUST, 1940. THE DATA HAVE BEEN MADE PROPORTIONATE BY RANDOM SUBSAMPLING

Specimen No.	No. of annulus					
	I		II		III	
	Area I	Area II	Area I	Area II	Area I	Area II
1	25.5	29.5	44.5	54.0	61.5	77.5
2	19.5	29.5	39.0	43.0	54.0	61.5
3	18.5	31.5	45.5	58.5	62.0	80.0
4	18.5	33.0	44.0	52.5	66.0	75.0
5	19.5	18.5	44.5	39.5	64.0	60.0
6	17.5	33.5	38.0	49.0	53.0	67.0
7	19.0	24.5	46.5	48.5	56.0	68.5
8	17.5	26.0	34.0	51.5	55.5	74.0
9	24.0	24.0	50.0	41.0	63.0	62.5
10	23.5	23.5	52.0	48.5	70.5	70.5
11	21.0	25.0	38.5	47.0	59.0	62.5
12	24.0	27.5	46.0	47.5	56.0	80.0
13	20.0	31.0	42.0	61.0	64.5	89.5
14	24.5	23.5	47.5	56.5	65.0	94.5
15	20.0	24.0	40.0	49.0	63.0	73.0

This problem was solved in the following manner: First, the largest scale from each "pectoral" scale sample (see Sampling Procedures) of these three-year-old bass was carefully mounted in a medium of white Karo syrup. Second, each of the mounted scales was projected on a ground glass screen at a fixed magnification of approximately 33 diameters. Third, a millimeter ruler was placed across, and through the center, of the projected scale image, i.e. from one side of the scale to the other. Fourth, the distance from the focus to each of the three annuli on the scale was read to the nearest millimeter. This meant that two measurements for each annulus were obtained, one on each side of the focus. Fifth, the two measurements for each annulus were averaged together and the result recorded in Table IX. And, sixth, the values in Table IX were transformed into two-place logarithms and the significance of difference between the annulus distances of the fish from the two areas was tested by means of an analysis of variance. The result is as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	89	3.5595	
Annulus groups	2	3.1480	1.5740
Area	1	0.1537	0.1537**
Annulus group-area interaction	2	0.0026	0.0013
Within subclasses	84	0.2552	0.0030

A highly significant difference having been demonstrated to exist between the means of the two area groups when considered collectively, there remains to be tested the significance of the difference between the means of the two groups when they are considered under each separate annulus group. This has been done by the method exemplified by Snedecor (8) for testing the significance of difference when only a single degree of freedom is involved. The results are as follows:

	Annulus group		
	I	II	III
Mean square $\frac{(S_1 - S_2)^2}{2k}$	.0908*	.0282*	.0441*

This analysis shows that a significant difference also exists between the means of the two area groups when any one of the three annulus groups is considered individually.

If, therefore, these annuli distances are reliable and comparable indicators of previous length increments in the fish, then significant differences between the lengths of these two groups of fish existed at one, two, and three years of age.

MISCELLANEOUS OBSERVATIONS

1. ANALYSIS OF STOMACH CONTENTS

Although qualitative and quantitative studies were made of the stomach contents of bass taken from the several areas throughout the summer of 1940, these will not be fully reported upon because of the highly doubtful value of the quantitative data. It was repeatedly observed, for example, that smallmouth bass, when hooked, would begin to regurgitate food; and if there was any greater tendency for the smaller particles, such as crayfish remains, to be retained—and this does not seem unlikely—the possible effects upon quantitative data are obvious. Also, the diurnal shifts in habitat already noted might well affect the stomach contents.

It may be stated, however, that during the summer of 1940, the smallmouth bass in Lake Simcoe apparently fed heavily upon crayfish and, perhaps to somewhat lesser extent, upon small fish, including: yellow perch (*Perca flavescens*); log perch (*Percina caprodes*); darters (*Boleosoma nigrum*); and sculpins (*Cottus bairdii*). It was interesting to note that despite the common occurrence of spot tailed minnows (*Notropis hudsonius*), and blunt nosed minnows (*Hyborhynchus notatus*) near the shoreline in Area I, these species were not found in the stomachs of those adult smallmouth that were examined. It seems possible, however, that the sublegal-sized bass might have been feeding upon these minnows.

2. PARASITISM

With a single exception, all those bass that were killed and retained showed the visceral adhesions generally associated with an infestation of bass tapeworm (*Proteocephalus ambloplites*). In most instances, plerocercoids could be found with but little difficulty and in many cases the infestation was extremely marked. It would be difficult to determine, of course, what effect this infestation has upon the fertility of the bass population in Lake Simcoe; but it should be emphasized that this parasite does not affect the health of man and that its effect upon the edibility of the fish is confined to possible objections upon aesthetic grounds.

In addition to the foregoing, three bass were taken during 1940 that showed lesions typical of lamprey attack.

3. WATER TEMPERATURES IN AREA I

Throughout the summer of 1940, bottom and surface temperatures were taken, from time to time, in the shelf region of Area I. On calm days, the bottom temperatures were always found to be slightly lower than surface readings. A slight chop, however, appeared to be all the agitation necessary to give similar temperatures at surface and bottom. These temperatures have been recorded in Table X.

TABLE X

BOTTOM TEMPERATURES, TO THE NEAREST HALF-DEGREE CENTIGRADE, TAKEN IN AREA I DURING THE PERIOD JUNE 14 to SEPT. 27, 1940

Date	Zones					
	I	II	III	IV	V	VI
	Temp.					
June 14	19.0					
June 17	12.0					
June 19	14.0					
June 21	9.5					
June 30	11.0					
July 1	13.0					
July 2	12.5					
July 3	15.0					
July 4	17.0	—	16.5	16.0		
July 5	17.5	17.0	17.0	17.0		
July 6	15.0	15.5	—	—		
July 7	18.0	—	—	—		
July 8	17.0	17.5	19.0	19.0	19.0	
July 9	18.5	—	—	—	—	
July 10	—	—	—	—	19.0	
July 12	—	17.0	17.0	—	—	
July 14	18.0	19.0	18.5	—	—	
July 16	—	—	20.0	19.0	19.0	
July 18	19.0	20.0	20.0	19.0	—	
July 20	20.5	21.0	20.5	—	—	
July 23	—	21.0	—	—	—	
July 24	—	26.0	25.5	25.0	24.5	
Aug. 1	—	22.0	21.5	—	—	
Aug. 7	—	23.0	23.5	—	—	
Aug. 10	—	—	—	22.0	—	
Aug. 19	22.0	—	22.0	—	—	
Aug. 20	21.0	—	21.0	21.0	21.5	21.5
Aug. 27	18.5	19.0	—	19.0	20.0	—
Aug. 31	19.0	—	—	—	—	—
Sept. 1	—	20.0	—	—	—	—
Sept. 19	17.0	18.0	18.0	—	—	—
Sept. 27	15.0	15.5	16.0	—	—	—

RESULTS OF ANGLING SINCE 1940

As indicated in earlier paragraphs, several persons who are either summer or permanent residents at Lake Simcoe have employed the trolling technique and procedure—sometimes with modifications such as substituting small plugs of the diving type—in Area I during all the summers since 1940. On occasion, guests of these persons, who were neophytes in the art of angling, have also used the described method. While no catch records have been kept on these subsequent angling efforts, the reports received demonstrate that the effectiveness of the trolling technique has continued to remain highly satisfactory for those who employ it. In short, the veriest tyro can look forward to worthwhile bass fishing in Lake Simcoe if he uses the method and procedures described herein.

Of additional interest are reports that the same zones in Area I may not produce the best angling returns from one year to the next. Further, that the number of sublegal-sized bass taken may also vary greatly from one year to another.

Comments and Conclusions

Upon the basis of the investigations and observations reported herein, it is concluded that:

(1) The smallmouth bass problem in Lake Simcoe is one of poor susceptibility and availability of the bass population to the methods of angling commonly employed in the lake.

(2) The developed angling methods herein described can successfully solve this susceptibility–availability problem.

(3) Shifts in habitat—both diurnal and otherwise— by adult smallmouth bass may affect the availability and susceptibility of these fish to capture by angling methods, and hence influence angling returns.

(4) Smallmouth bass in the northern section of Lake Simcoe designated as Area II had a significantly faster rate of growth than bass in the Kempenfeldt Bay region designated as Area I; and this may affect the quality of catch.

(5) Little—if any—difference in rate of growth exists between male and female smallmouth bass in Lake Simcoe.

(6) The great majority of adult smallmouth bass in Lake Simcoe were, during 1940, infested with bass tapeworm, *Proteocephalus ambloplites*.

(7) The adult smallmouth bass in Lake Simcoe feed largely upon crayfish and upon young fish including *Perca flavescens*, *Percina caprodes*, *Boleosoma nigrum*, and *Cottus bairdii*. For a short period during early summer they also feed upon adults of the mayfly, *Hexagenia* sp.

(8) Sufficient evidence is not yet at hand for accurately predicting the occasional shifts in habitat that are made by the adult portion of the bass population. The existing evidence suggests, however, that there is a seasonal shift in habitat near midsummer and, sometimes, a return during late summer. This is suggested not only by the evidence as reported herein, but also by the fact that certain professional guides at the northern part (Area II) of Lake Simcoe often troll for bass during the early part of summer, in regions and habitats that are seldom fished after midsummer.

(9) Of equal interest is the evidence of late evening shifts in habitat by the adult bass. These late evening shifts are encountered in both Area I and Area II. In this latter Area, for instance, an extremely effective procedure for taking bass is to employ a bait method in certain "spots" on the shoals during the day, and troll the shallow waters of the shoals during the evening. It is necessary, however, to discover where some of these daytime "spots" are situated; and this can often be done by canvassing the shoals with the trolling method during the day for indications of available fish.

(10) In view of the paucity of bass anglers in Lake Simcoe—one seldom encounters a half-dozen outside of Area II—it would appear that a much

fuller utilization of the resource could be best accomplished through dissemination of knowledge on the improved methods of angling and on the habits of the fish as reported herein.

Acknowledgments

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A REDESCRIPTION OF *CEPHALOBELLUS BREVICAUDATUM* (LEIDY, 1851) CHRISTIE, 1933 (NEMATODA), WITH COMMENTS ON OTHER SPECIES OF THE GENUS *CEPHALOBELLUS*[1]

BY M. A. BASIR[2]

Abstract

A complete redescription of *Cephalobellus brevicaudatum* (Leidy, 1851) Christie, 1933, is given; its specific relationship is discussed, and a key to the species of the genus *Cephalobellus* is given. It is proposed that *Thelastoma indiana* Basir, 1940, and *Cephalobellus lloydi* Baylis, 1946, be regarded as synonymous with *C. brevicaudatum.*

In 1851 Leidy (8) described *Thelastoma brevicaudatum*, a new species of nematodes from the larvae of a "large lamellicorn insect." He gave no figures and his description was brief. He gave the following diagnosis:

> "Body white, cylindrical, anterior to commencement of ventriculus rapidly narrowed, conoidal; posteriorly abruptly rounded, with a very short spiculate tail. Generative aperture just posterior to middle. Anus just anterior to posterior rounded termination. Ventriculus broadly dilated at commencement, and moderately so at termination.
>
> "Length of body 1¼ to 2 lines (=2.642-4.233 mm.); anterior to ventriculus 1/50 inch (=508μ); from anus to end of tail 1/200 inch (=127μ); at middle 1/80 inch (=317μ); at commencement of ventriculus 1/75 inch (=312μ); cylindrical portion of oesophagus 1/66 inch (=385μ) long by 1/666 inch (=51μ) broad; pyriform portion 1/250 inch (=101μ) long by 1/285 inch (=89μ) broad. Ovum semi-oval, 1/285 inch (=89μ) long by 1/1000 inch (=25μ) broad."

Later in the same year he (9) subdivided the genus *Aorurus* Leidy, 1849, into two subgenera, *Streptostoma* and *Thelastoma*, thus placing all the species previously described under the generic name *Thelastoma* into the genus *Aorurus*. *Thelastoma brevicaudatum* then became *Aorurus* (*Thelastoma*) *brevicaudatum.*

In 1853 the same author (10) raised the subgenera *Streptostoma* and *Thelastoma* to generic rank as *Streptostomum* and *Thelastomum*, and again gave a brief description of this species as *Thelastomum brevicaudatum.*

In the course of a synopsis of the parasites described by him up to that time, Leidy (11) placed this species as a queried synonym of *Thelastomum robustum*

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Leidy, 1850. Diesing (6) makes *Thelastoma* a subgenus of the genus *Anguillula* and calls *T. robustum*, *Anguillula (Thelastoma) robusta*, while retaining *T. brevicaudatum* as a synonym of *T. robustum*.

Travassos (12) suppressed the genus *Streptostomum* and revived the genus *Aorurus*, most probably on priority reasons. Walton (14) in his revision of the nematodes of the Leidy collections again revived the classification of Leidy (1851) and subdivided the genus *Aorurus* into two subgenera, *Streptostoma* and *Thelastoma*, as Leidy had done. Thus he again named this species *Aorurus (Thelastoma) brevicaudatus* (Leidy, 1851). Travassos (13) recognizes *Thelastoma* and *Aorurus* as distinct genera and calls this species by the name of *Thelastoma brevicaudatum* Leidy, 1851, as originally described by Leidy.

Christie (3) described *Scarabanema cylindricum* n.g., n.sp., from the larvae of several scarabaeid beetles. He removed *Thelastoma brevicaudatum* from the genus *Thelastoma* and placed it in his new genus as *S. brevicaudatum* n. comb. He also placed in his new genus, *Thelastoma leuckarti* (Hammerschmidt, 1838 (7)) Travassos, 1929, as *S. leuckarti* n. comb. Christie quotes the diagnosis of *S. brevicaudatum* from the original by Leidy and, in differentiating it from his species, remarks: "The above diagnosis taken from Leidy's original description indicates that this parasite belongs to the genus *Scarabanema*, but it is scant information upon which to identify the species." Later, in 1933, he (4) made his genus *Scarabanema* a synonym of the genus *Cephalobellus* Cobb, 1920, as he found that there was no difference between the male of *S. cylindricum* and *C. papilliger*. Cobb (5) has only given the description of the male worm unaccompanied by any figures. In 1946 Baylis added another species to this genus and named it *C. lloydi*.

The present writer recovered from a cockroach some worms, which appear to be identical with those described by Leidy as *C. brevicaudatum* (= *Thelastoma brevicaudatum*). As Leidy had given a very brief and incomplete description and had given no figures at all, it seems appropriate to take this opportunity to describe the species fully. No other worker, to the knowledge of the writer, has redescribed this species since Leidy, although Walton (14) reported that he had seen this form.

Cephalobellus brevicaudatum (Leidy, 1851) Christie, 1933

Synonyms:

Thelastoma brevicaudatum Leidy, 1851;
Aorurus (Thelastoma) brevicaudatum Leidy, 1853;
Thelastomum brevicaudatum Leidy, 1853;
Aorurus (Thelastoma) brevicaudatus (Leidy, 1851) Walton, 1927;
Scarabanema brevicaudatum (Leidy, 1851) Christie, 1933.
Thelastoma indiana Basir, 1940.
Cephalobellus lloydi Baylis, 1946.

Diagnosis: Cephalobellus

Male: Not found by the writer.

Female (Fig. 1): Body cylindrical, tapering more or less suddenly anterior to the base of the oesophagus, giving a conoid shape to the anterior end of the worm; posteriorly it tapers to form a short conoid spiculate tail. It is 2.2 to 2.6 mm. long by 250μ in maximum width. The cuticle is transversely striated throughout the whole length of the body except in the tail. The first annule behind the head is wider than the rest and is 13μ wide. The remaining annules, up to the middle of the body, are 8 to 12μ apart. In the posterior region of the body they are larger and reach a width of up to 15μ.

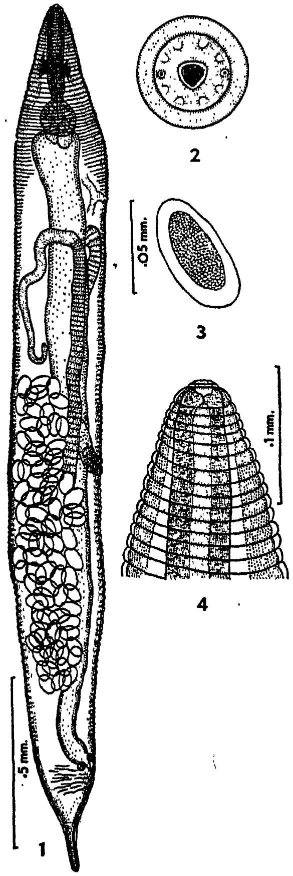

FIG. 1. *Female, entire lateral view.* FIG. 2. *Female, en face view.*
FIG. 3. *Egg.* FIG. 4. *Female, anterior region.*

The oral opening is subtriangular and is surrounded by a circumoral elevation and eight papillae (Fig. 2). Amphids are present laterally as very small openings. The buccal cavity is short and is 8μ long by 8μ wide (Fig. 4).

The oesophagus nearly reaches the anterior extremity. It is 350 to 390μ long and consists of a more or less cylindrical corpus, 240 to 265μ long by 40 to 50μ wide, a short but distinct isthmus, 20μ long by 25 to 35μ wide, and a posterior valvular bulb 90 to 105μ long by 90 to 110μ wide. The oesophagus communicates with the intestine through a pair of well developed oesophagointestinal valves. The intestine is enlarged anteriorly to form a slight cardia. It also shows a posterior enlargement just before opening into the rectum. The rectum is short. Two rectal glands are present. The anus is situated 260 to 280μ from the posterior end of the body. The body suddenly tapers behind the anus and is drawn out into a short, blunt terminal spike.

The excretory pore lies posterior to the base of the oesophagus, about 600μ from the anterior end of the body. The nerve ring lies at about two-thirds of the length of the corpus from its anterior end, between 150 and 180μ from the anterior extremity.

The reproductive system is double. The vulva lies at a distance of 1.4 to 1.48 mm. from the anterior end of the body, 58 to 60% of the body length from the anterior end. The vagina is tubular and muscular and is directed anteriad. There are two ovaries. The anterior ovary is reflexed at a distance of about 280μ posterior to the base of the oesophagus and posteriorly the reproductive organs extend up to 250μ from the anus. The eggs are oval to elongated oval, 60 to 75μ long by 25 to 35μ wide, not segmented at time of deposition (Fig. 3).

Host: *Leucophoea* sp. (Family Blattidae).

Location: Intestine.

Distribution: North India; U.S.A. (near Philadelphia, Pa.).

Discussion

Those who have worked with the nematodes of the family Thelastomatidae know well that the characters of the male, which form the basic means for identifying genera and species of other groups, cannot be used for such a purpose in this group. Firstly, males are very rare and secondly, when they are found, there is often present a pronounced sexual dimorphism, so that in cases where two or more species inhabit the same host it is difficult to match the right males and females. Therefore, the basis of taxonomy of the Thelastomatidae rests mainly on the characters of the females.

Baylis (2) described *Cephalobellus lloydi* n. sp., from dipterous larvae (larvae of tipulids, crane-flies or "daddy longlegs") possibly belonging to *Tipula peliostigma* Schummel from Berkshire, and *Tipula oleracea* L. from Yorkshire (England). He compares his species with *C. papilliger*. The males he has described are almost identical with those described by Christie for the latter species, but his females are different from the females of that species in that in *C. papilliger* the vulva lies at about 52% of the body length from the anterior end, and the eggs are subspherical to oval, 70μ long by 45μ wide; in *C. lloydi* the vulva is situated at about 61% of the body length from the anterior end, and the eggs are more than twice as long as broad, and are 82 to 96μ long by 32 to 38μ wide. In both these characters *C. lloydi* comes very close to *C. brevicaudatum*. The vulva in the latter lies between 58 and 60% of the body length from the anterior end and the eggs are 89μ long by 25μ wide. Baylis, in his discussion, writes: "The vulva, however, is stated by Leidy to be just posterior to the middle of the body, and this appears to differentiate the species from *C. lloydi*." As a matter of fact, Leidy did not give the exact position of the vulva. Now, in this present redescription of Leidy's species, the writer finds that the vulva lies at 58 to 60% of the body length from the anterior end. Thus this difference, on which Baylis mainly created his species, does not exist. The only difference now left is in the size of the eggs but if all the measurements, that is, those of Leidy, of Baylis, and of the writer are examined, it will be noticed that the sizes merge into each other.

Another difference, which Baylis points out, is in the respective hosts. Leidy found his worms in scarabaeid larvae. The worms described by Baylis were recovered from dipterous larvae while the writer obtained his from a cockroach. As the specificity of nematodes of arthropods has not yet been studied, it cannot be said that specific distinctions may be made on host relationship only, as has been done for forms like the different species of the genus *Strongyloides*.

As the morphology of both the worms is the same, the writer, after the present redescription, therefore, believes that *C. lloydi* Baylis, 1946, is synonymous with *C. brevicaudatum* (Leidy, 1851) Christie, 1933.

Thelastoma indiana Basir, 1940 (1), described from a cockroach, is also identical with *C. brevicaudatum* and, therefore, becomes a synonym to the latter species.

Thus the genus *Cephalobellus* Cobb, 1920, at present contains three species only, namely, *C. papilliger* Cobb, 1920; *C. leuckarti* (Hammerschmidt, 1838) Christie, 1933; and *C. brevicaudatum* (Leidy, 1851) Christie, 1933.

A revised key to these species is given below.

KEY TO THE SPECIES OF THE GENUS *Cephalobellus*

(1) Vulva almost in middle of body; eggs less than twice as long as broad............. 2

Vulva about 60% of the body length from the anterior end; eggs almost twice or more as long as broad...*C. brevicaudatum*

(2) Eggs subspherical, 88–110μ long by 73μ wide; female with three conspicuous yellowish bodies near vagina...*C. leuckarti*

Eggs smaller, oval, 70μ long by 45μ wide; females without any yellowish bodies near vagina...*C. papilliger*

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THE INFLUENCE OF GONADAL HORMONES ON THE SERUM VITAMIN A OF THE IMMATURE PULLET[1]

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Abstract

The serum vitamin A level of sexually immature pullets was increased threefold to fourfold by intramuscular injection of 24 mgm. estradiol dipropionate plus 4.5 mgm. testosterone propionate administered in six equal doses over a 12-day period as compared with the level in similar pullets receiving only testosterone propionate. It is suggested that gonadal hormone activity is one of the factors that regulate serum vitamin A levels in the domestic fowl.

Introduction

Administration of estrogens to sexually immature pullets or cockerels produces profound changes in blood composition. The resultant increases in serum calcium, serum inorganic phosphorus, serum phospholipid, and serum phosphoprotein have been extensively studied. The earlier literature has been reviewed by Riddle (9). Serum neutral fat and cholesterol are also increased (6), while there is some evidence that serum citric acid is decreased (3). Serum riboflavin is greatly increased (1), while the increase in blood volume appears to be due to an increase in plasma without a corresponding increase in volume of blood cells (2). There does not appear to be any extensive information as to the effects of estrogen on serum vitamin A in the fowl. Williamson (11) records that injections of estradiol benzoate decreased the serum vitamin A of rabbits. Gonadal hormone activity in the pullet as it comes into lay, however, almost certainly involves androgenic as well as estrogenic activity. The most obvious signs are the reddening and hypertrophy of comb and wattles that take place during the period of 10 to 14 days before laying. Treatments designed to simulate gonadal hormone activity in the pullet as it passes into the reproductive stage will therefore include an appropriate amount of androgen as well as estrogen.

So far as previous work goes it seems that estrogen alone will produce changes that simulate the relevant changes in serum calcium, serum phosphorus, and serum riboflavin. The magnitude of the changes evoked by estrogen in respect of these blood components is not affected by concurrent administration of androgen in amounts designed to cause changes simulating the relevant comb and wattle changes (1, 3, 4). Such concurrent administration of androgen does, however, enhance the effect of estrogen upon hypertrophy of the oviduct (1, 3, 4). Concurrent administration of androgen also enables estrogen to increase the rate of calcium retention so as to simulate

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the increase in rate of calcium retention that precedes the normal beginning of laying (3, 4).

In the experiments now reported attention was restricted to the investigation of the effects on serum vitamin A of different levels of estrogen given concurrently with a single level of androgen. This inclusion of androgen was designed to secure a closer simulation of normal gonadal hormone activity than that obtained by estrogen treatment alone.

Experimental

Experiment 1

Eight Barred Plymouth Rock pullets of the same strain and hatching were placed in individual cages. They received a commercial starting mixture. Daily food consumption was kept equal as between the different birds. This point demands close attention in such experiments because of the stimulating effect of gonadal hormones on appetite. If fed *ad libitum*, then estrogenized birds consume more food and there is uncertainty as to the extent to which differential food consumption may be affecting the results.

The hormonal treatments were given in sesame oil solution by intramuscular injection into the breast muscles. Care was taken to ensure that the same total amount of sesame oil was injected into each bird.

Each treatment was divided into six equal doses administered on alternate days. All the pullets were treated with testosterone propionate (Schering) in amounts designed to secure a degree of comb hypertrophy comparable with that which takes place as the pullet comes into egg production. In addition graduated doses of estradiol dipropionate (Schering) were given as outlined in Table I.

TABLE I

EXPERIMENT 1. EFFECT OF GONADAL HORMONES ON SERUM VITAMIN A OF IMMATURE PULLETS

Pullet No.	1739	1743	1740	1744	1741	1745	1742	1746
Total dosage of estradiol dipropionate, mgm.	Nil	Nil	6	6	12	12	24	24
Total dosage of testosterone propionate, mgm.	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Live weight, initial, kgm.	1.12	0.99	1.05	1.00	1.06	1.15	1.24	1.10
Live weight, final, kgm.	1.27	1.09	1.23	1.16	1.18	1.23	1.34	1.21
Total food for experimental period, kgm.	0.83	0.83	0.84	0.85	0.84	0.84	0.84	0.84
Oviduct weight, gm.	0.24	0.30	13.9	16.2	17.0	20.9	22.4	17.2
Serum calcium, mgm./100 ml.	13.0	13.7	32.7	34.3	82.5	82.0	118.0	119.0
Crude blood volume, ml.	31.0	28.0	31.0	41.0	52.0	53.5	58.5	59.0
Crude blood volume, ml./kgm. live weight	24.4	25.7	25.2	35.4	44.1	43.5	43.6	48.8
Crude serum volume, ml./kgm. live weight	14.1	15.4	14.9	25.1	33.8	33.2	33.3	38.5
Serum vitamin A, I.U./100 ml.	153	119	170	282	320	499	528	602
Serum vitamin A, I.U./kgm. live weight	22	18	25	66	106	169	176	231

The birds were decapitated and bled on the second morning after the sixth injection. The blood was collected, allowed to clot, and the volume recorded as "crude blood volume". The "crude serum volume" per kgm. live weight was calculated on the assumption that about 10.3 ml. cells per kgm. live weight will be obtained from such birds by free bleeding (2). The figures secured in this way are approximations, but they make it possible to arrive at some estimate of the total serum vitamin A per kgm. live weight.

Serum calcium was determined by Halverson's method (8). Serum vitamin A was estimated by the method of Dann and Evelyn (5), except that it was found necessary to use twice as much alkali in the initial saponification because of the higher protein content of the sera of estrogenized birds. The "L" values obtained with the Evelyn photoelectric colorimeter were converted to E values by using the factor 0.50 as determined by McFarlane and Sutherland (7). The E values were converted to I.U. vitamin A using the conversion factor 1894.

The blue values in the antimony trichloride reaction were corrected for carotene as described by Dann and Evelyn (5), but in all the experiments now described this correction was very small and could have been omitted without appreciably affecting the results.

The birds were 82 days old at the beginning of the injections and 94 days old when killed.

The data are summarized in Table I. The degree of hypertrophy of the oviduct and the increase in serum calcium and blood volume with increasing dosage of estrogen are similar to those recorded elsewhere for similar pullets (2, 4). There was not any evidence of ovarian activity.

The level of serum vitamin A was increased about fourfold by the heaviest estrogen treatment. The smaller treatments gave intermediate increases. The relative increase in serum vitamin A is considerably greater when considered on the basis of total serum vitamin A per kgm. live weight.

Experiment 2

This experiment was a repetition of Experiment 1, excepting that (*a*) three pullets were placed under each treatment and (*b*) the commercial starting mixture was replaced by the following mixture.

Ground wheat	25 lb.
Ground corn	25 lb.
Ground barley	25 lb.
Wheat middlings	5 lb.
Soybean oil meal	15 lb.
Fish meal	2.5 lb.
Salt	1.0 lb.
Calcium carbonate	2.0 lb.
Fortified fish oil (2200 I.U. vitamin A per gm.)	0.25 lb.
Manganese sulphate	6 gm. per 100 lb. mixture

The pullets were 84 days old at the beginning of the injections and 96 days old when killed.

The average results are given in Table II. It will be noted that they confirm the results secured in Experiment 1.

TABLE II

EXPERIMENT 2. EFFECT OF GONADAL HORMONES ON SERUM VITAMIN A; AVERAGE RESULTS FOR THREE PULLETS ON EACH TREATMENT

Total dosage estradiol dipropionate, mgm.	0	6	12	24
Total dosage testosterone propionate, mgm.	4.5	4.5	4.5	4.5
Live weight, initial, kgm.	0.98	0.92	1.00	1.04
Live weight, final, kgm.	1.17	1.11	1.19	1.15
Total food consumption, kgm.	0.72	0.76	0.74	0.72
Oviduct wt., gm.	0.26	17.8	18.8	22.6
Serum calcium, mgm./100 ml.	12.9	37.4	90.6	114.7
Crude blood volume, ml.	35	32	52	71
Crude blood volume, ml./kgm. live weight	30	29	47	59
Crude serum volume, mgm./kgm. live weight	29	25	37	49
Serum vitamin A, I.U./100 ml.	158	217	342	413*
Serum vitamin A, I.U./kgm. live weight	32	42	127	196*

Omitting anomalous very low result for one serum.

Experiment 3

A third experiment was carried out using 12 pullets, six of which received no estrogen and six of which received 18 mgm. estradiol benzoate (Schering). The birds received the same mixture as was fed in Experiment 2. The blood from one bird was accidentally lost. The average results for the other 11 pullets are set out in Table III.

TABLE III

EXPERIMENT 3. EFFECT OF GONADAL HORMONES ON SERUM VITAMIN A

	Group *A*	Group *B*
No. of pullets	5	6
Total dosage estradiol benzoate, mgm.	0	18
Total dosage testosterone propionate, mgm.	4.5	4.5
Live weight, initial, kgm.*	0.94	0.94
Live weight, final, kgm.*	1.11	1.09
Total food consumption, kgm.	0.78	0.79
Oviduct wt., gm.	0.22	14.8
Serum calcium, mgm./100 ml.	12.8	100.1
Crude blood vol., ml.	39.2	59.5
Crude blood vol., ml./kgm. live weight	35.8	44.6
Crude serum vol., ml./kgm. live weight	25.5	34.3
Serum vitamin A, I.U./100 ml.†	146	382
Serum vitamin A, I.U./kgm. live weight**	37	170

* *Difference between groups not significant.*

** *Difference between groups highly significant at 1% level ("t" = 8.65).*

† *Difference between groups highly significant at 1% level ("t" = 7.16).*

The results leave no doubt that estrogen treatment superimposed on a small testosterone treatment increased the level of serum vitamin A.

Discussion and Summary

The levels of serum vitamin A observed in the unestrogenized pullets in these experiments fall well above the level of 50 to 75 I.U. per 100 ml. suggested by Taylor *et al.* (10) as representing marked depletion of body reserves. They are, in fact, of the same order of magnitude as the values found by Taylor *et al.* in the sera of hens receiving a diet judged adequate in provitamin A.

The increase in serum vitamin A that followed treatment with estrogen was not a consequence of increased food intake. The extra vitamin A must, therefore, have been obtained either (*a*) by increased retention from the food or (*b*) by depletion of body reserves or deflection of some absorbed vitamin A from storage. Taylor *et al.* (10) comment upon the variability of the serum vitamin A levels observed by them in laying hens receiving adequate provitamin A. It is possible that this variability is to some extent at least an expression of variability in endogenous estrogen production, which may control the distribution of vitamin A between liver reserves and the blood serum.

The present experiments leave little doubt that treatment with estrogen in conjunction with androgen increases serum vitamin A in the immature pullet. It seems reasonable to infer that the increase of endogenous estrogen and androgen production as the pullet comes naturally into reproduction will tend to affect serum vitamin A in the same sense.

Acknowledgments

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THE STRUCTURE OF THE ASCIDIAN, *PYCNOCLAVELLA STANLEYI* N. SP., AND THE NATURE OF ITS TADPOLE LARVA[1]

By N. J. Berrill[2] and D. P. Abbott[3]

Abstract

A new species of an ascidian, *Pycnoclavella stanleyi*, is described. It occurs in some abundance near low tide level at Pacific Grove, Calif. It differs from *P. aurilucens* Garstang, known only from the western end of the English Channel, mainly in the character of the colony rather than that of the individual zooid or tadpole larva. Colonies are flat, incrusting, heavily impregnated with sand, have a gelatinous matrix, and exhibit some orange pigmentation. Colonies of *P. aurilucens* are without sand, have a leathery texture, are green, and are not intertidal. Both species have zooids with seven rows of gill slits on each side, a posterior vascular budding stolon as in *Clavelina*, and a tadpole larva with an ocellus but not an otolith.

In his account of the ascidian *Euherdmania claviformis*, Van Name (9) states that "at various points on the California coast (at Pacific Grove and Bodega Bay) there may be found among the roots of seaweeds near low-water mark, where sand as well as rocks are present, dense sandy masses of small tubular zooids closely packed, the masses often 6 to 10 cm. across, which appear to be colonies of young zooids of this species." He found the colonies to be immature and doubted that they could be a form allied to *E. claviformis* but with smaller zooids, yet felt that the matter deserved further study.

Collections of ascidians have recently been made by one of us (D.P.A.) in the region of Pacific Grove. Ascidian colonies with the general appearance of those described by Van Name were found at Aselomar Rocks and at other points. Some of these contained zooids with but three rows of gill slits and appear to be colonies of *Eudistoma ritteri* Van Name. Other colonies had zooids with seven rows of gill slits, a number that excludes them from the genus *Eudistoma* and almost as certainly from *Euherdmania* as well. When the character of the gills is taken together with other characters, no doubt is left that the colonies are a species of the genus *Pycnoclavella*, established by Garstang in 1891, and that the species is new. It is a pleasure, therefore, of the senior author to name it *Pycnoclavella stanleyi* after his colleague, John Stanley. This accordingly becomes the second species of the genus, the other and type species being *Pycnoclavella aurilucens* Garstang (8), recorded with certainty only near Plymouth, from the Mewstone ledge in the English Channel; the structure and life cycle of *P. aurilucens* have been recently described elsewhere (1).

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Contribution from the Department of Zoology, McGill University, Montreal, Que., and the Department of Zoology, University of California, Berkeley, Calif.

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Pycnoclavella stanleyi n. sp.

The colonies grow on rocks or at the base of seaweeds near the low-water mark, forming flat masses several centimeters across and about 1 cm. thick. Except for the thoracic region, individual zooids are usually embedded in the common test, which is comparatively soft, and the thoracic part of each zooid extends freely from the exposed surface. Each colony examined showed some evidence of being superimposed upon hydroid creeping stolons, and it is possible that these are a factor in its initial establishment. Two superficial characters are distinctive. The matrix of the colony is impregnated and covered with sand grains of all kinds, together with small worm tubes and calcareous algae, making observations of the basal regions most difficult, while the emergent thoracic regions are typically a bright orange, a color that fades upon preservation to a dull brown. The orange color is due to special cells scattered through the test surrounding the free part of each zooid outside the epidermis. The function of these cells is obscure.

Structure of zooid

The general morphology of the zooid is shown in Fig. 1. The zooid is divisible into a thorax and abdomen, with an attenuated oesophageal region between. There is no true postabdomen as in *Euherdmania*. Each zooid, being an independent unit, has separate branchial and atrial siphons. There is no arrangement of zooids into systems and consequently no corresponding modification of the atrial siphon. Both siphons are smooth-edged, as in *Clavelina* and *Euherdmania*, and not divided into six lobes as in *Eudistoma*.

The branchial sac has seven rows of stigmata on each side, in contrast to the three rows of *Eudistoma* and 10 rows of *Euherdmania*. The abdomen is long and slender, with the digestive tube descending to its posterior limit. The digestive tube itself consists of a long narrow oesophagus opening posteriorly into a more or less globular stomach in the lower part of the abdomen, a mid-intestine forming the posterior loop proper, connecting the stomach with the long ascending rectum, which opens at the base of the atrial cavity (Fig. 1A). The rectum is commonly distended like a string of beads by the large round faecal pellets formed in passage through the stomach.

The heart and pericardium lie alongside the posterior loop of the intestine, and has one end opening into the ventral vessel that extends forwards eventually to pass along the ventral side of the endostyle, and posteriorly to open towards the posterior vascular extension (Fig. 1A, *pv*). This last structure is usually short and narrow and contains an internal vascular septum separating the afferent and efferent flow of the blood. At the junction of this extension with the abdomen are three corrugated protrusions representing the posterior insertions of the longitudinal muscles of the body.

The epicardium, i.e. the closed membranous sac adjoining the digestive tube, of possible excretory function, extends as a simple single sac flattened dorsoventrally, from just below the thorax to the lower part of the heart, at the ventral side of the digestive tract (*cp*, cross sections shown in Fig. 1, B, C, and D).

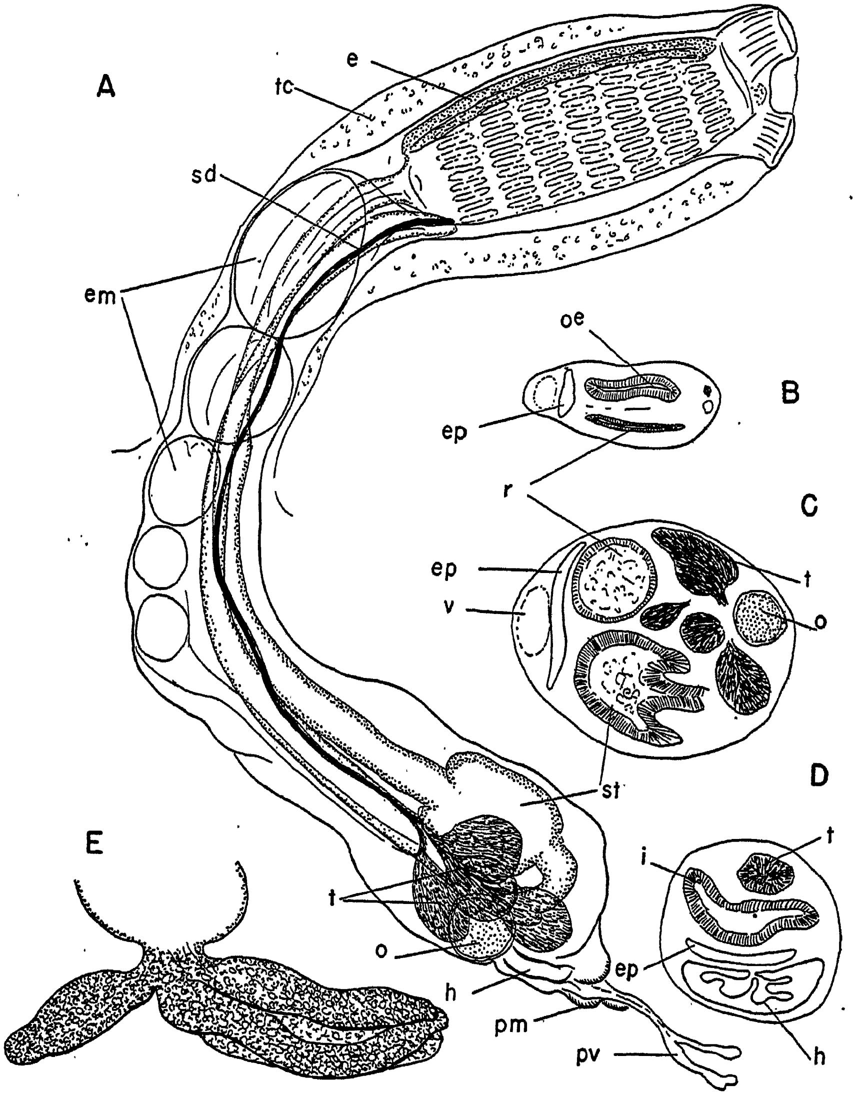

FIG. 1. *Structure of zooid of Pycnoclavella stanleyi.*

A, complete zooid showing thorax with independent siphons and seven rows of gill slits, oesophageal region with developing embryos, and abdomen with stomach, gonads, and heart. B, C, D, cross sections of zooid at level of oesophagus, stomach, and heart, respectively. E, posterior end of zooid in accumulation phase, with posterior vessels distended with reserve cells.

e, endostyle; em, embryos; ep, epicardium; h, heart;, i, loop of intestine; o, ovum; oe, oesophagus; pm, posterior muscle insertions; pv, posterior vessel; r, rectum; sd, sperm duct; st, stomach; t, lobe of testis; tc, test cell; v, ventral vessel.

The gonads lie in the abdomen at approximately the same level as the stomach and consist of an ovary and several testicular lobes, the oviduct and common sperm duct following the course of the rectum to open at the base of the atrial cavity.

Eggs are passed from the ovary one at a time into the oviduct as they ripen and are there fertilized, mature zooids like the one illustrated usually possessing a series of embryos developing within the oviduct, which thus serves as a brood chamber.

Budding

The only colonies available for examination were collected in July during the sexual breeding period, and ascidian colonies in general rarely exhibit budding processes and sexual reproduction simultaneously. At the same time two colonies showed an unusual condition at their base. The posterior region of the zooids of these colonies was extended and distended, in the majority involving the posterior vascular extension only, in some the posterior end of the abdomen including the longitudinal muscles. A typically inflated region is shown in Fig. 1E. The vascular processes especially are distended with reserve cells. These are large cells heavily laden with several large pseudo-yolk granules and corresponding to the trophocytes of *Clavelina* (7) and *Diazona* (4). These cells are to be found scattered through the body of the zooid and they migrate posteriorly and progressively accumulate in the lower part of the abdomen. With dilatation of the posterior extremity and vascular processes, they pass into the processes to form massive accumulations of reserve material. They appear to be similar to, and possibly may be identical with, the orange cells of the thoracic test.

The structure of the zooid is such that only two methods of budding appear to be possible. The abdomen, including the oesophageal region, may undergo transverse epidermal constrictions to form several buds, as in *Eudistoma* and *Archidistoma* (2, 3), or the zooid as a whole may regress, leaving the congested posterior vascular processes to give rise to new zooids as in species of *Clavelina* (6) and as is probable in *Pycnoclavella aurilucens* (1). The latter is much the more likely, for it is only in those forms in which the reserve cells congest the abdomen that abdominal buds are formed. When the reserve cells can pass posterior to the abdomen into either distended vascular processes or into a true postabdomen, both abdomen and thorax undergo regression, and since this species allows this passage to take place, this probably occurs.

Nature of Tadpole Larva

The structure of the tadpole is shown in Fig. 2. The fully formed tadpole (Fig. 2A) is relatively large, with an ovoid trunk and comparatively long tail. The tail is twisted at its base so that its axis is at right angles to the axis of the trunk. There are the usual three anterior adhesive organs (Fig. 2A, *ad*), while the tail structure is primitive, with three rows of muscle cells along each side of the central notochord (Fig. 2E). The primary ascidiozooid is well formed within the trunk and has its main anteroposterior axis already at right angles to that of the tadpole itself.

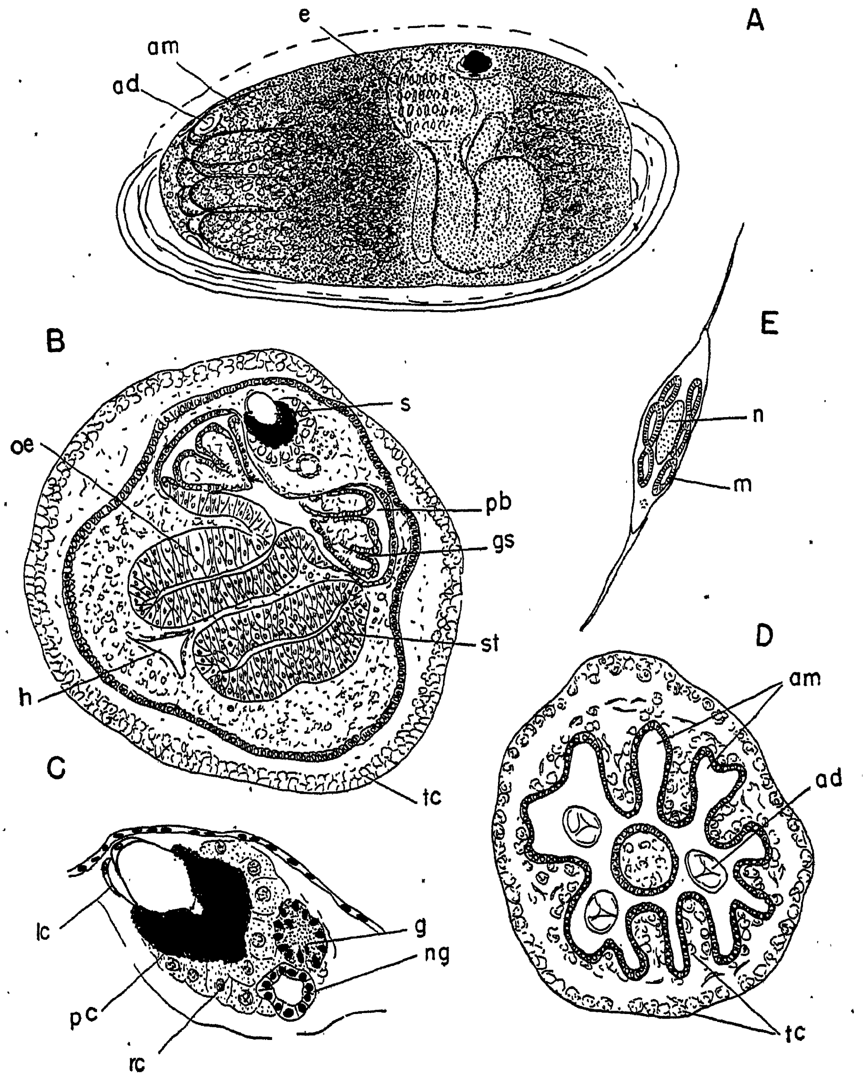

FIG. 2. *Tadpole larva of Pycnoclavella stanleyi.*

A, whole tadpole with tail coiled, showing single sense organ, anterior ampullary structure, and central ascidiozooid with four rows of gill slits. B, transverse section through level of sense organ. C, sense organ enlarged to show optic cup and lens cells. D, transverse section through anterior ampullae. E, transverse section of tail.

ad, adhesive organ; am, ampulla; e, endostyle; g, neural ganglion; gs, gill slit; h, heart; lc, lens cell; m, tail muscle cell; n, notochord; oe, oesophagus; pb, peribranchial cavity; pc, pigment cup; rc, retinal cell; s, sense organ; st, stomach; tc, test cells.

There are, however, three distinctive features. The test substance surrounding the trunk is heavily impregnated with large pigmented test cells derived from the tadpole and not from the parent zooid (Fig. 2B, D, *tc*). The anterior epidermis of the tadpole is developed to form about eight ampullary divisions (Fig. 2A, D, *am*), while the sensory vesicle contains an ocellus only, there being no trace of an otolith.

There is virtually no free space within the sensory vesicle. The neural ganglion and gland of the future zooid are already well developed (Fig. 2C, *g*, *ng*). The ocellus itself consists of a typical pigment cup partly surrounded by a single layer of large retinal cells, and two large lens cells (Fig. 2C, *lc*).

Discussion

This species shares with *Pycnoclavella aurilucens* the following features,—thorax of zooids extending freely from a common matrix, siphons smooth-edged, branchial sac with seven rows of gill slits on each side, no postabdomen, posterior vascular processes becoming distended with reserve cells, embryos developing in series within oviduct, and tadpole larva with ocellus but no otolith. These features in common are more than sufficient to unite the two forms as species of the same genus, and the absence of an otolith in the tadpole becomes here a generic and not a specific character.

Euherdmania zooids have 10 or more rows of gill slits, a true postabdomen containing heart and gonads, a uniquely double epicardium, and a tadpole with both ocellus and otolith (5). There is consequently no possibility that we are dealing with a species of this genus.

The differences in the two colonies refer mainly to details of the common test or matrix in which the zooids are embedded. In *P. aurilucens* the test cells are green instead of orange and there is no tendency to incorporate sand grains in its comparatively leathery test. The tadpoles differ in one feature only, namely, the presence of the ampullary organization of the anterior trunk epidermis of *P. stanleyi* and the absence of this in *P. aurilucens*. Anterior ampullae are so frequent among the various ascidian tadpoles that it is hardly distinctive, though in this case it does suggest some affinity with the *Archidistoma–Eudistoma* forms rather than with *Clavelina* itself.

The distribution of these two species is of interest. Each is known only from a very limited locality, for there is no record of *P. aurilucens* other than from one or two localities in the western end of the English Channel, while *P. stanleyi* is known from an equally limited region of the North American Pacific coast.

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THE TWO TYPES OF EPITHELIUM OF THE FINEST BRONCHIOLES OF THE ALBINO MOUSE AS REVEALED BY SUPRAVITAL SILVERIZATION[1]

By Charles C. Macklin[2]

Abstract

Two definite types of cells, provisionally designated dark and light, in accordance with the degree of their superficial argyrophilism, are revealed in the terminal bronchiolar epithelium of the albino mouse by supravital silverization. The dark cells are ciliated, and scattered among the light unciliated cells as singles, pairs, triads, or larger groups to form a striking and characteristic surface pattern. The cuticles of the dark cells, far outreached by the neighboring light cells, appear *en face* as dense crowds of small uniform golden brown granules. Viewed laterally these show as pairs in vertical alignment, and make two layers with a thin lighter band between. The sides and bases are indicated by deposits of black grains. Near the sharply marked margin of the definitive epithelium the dark cells are shorter, more dispersed, and formed like truncated pyramids with narrow densely impregnated apices on some of which are single delicate points instead of discrete cilia. The light cells have bulging villuslike free ends and make flutings on the contiguous dark cells. Small silverized particles sparsely stipple their air surfaces and rounded plaques crown their summits. A similar dicellular picture is found in the terminal bronchiolar epithelium of the golden hamster.

Introduction

The epithelium of the last quarter millimeter of the purely conducting bronchioles (Macklin, 1929 (4)) is largely unexplored. In an attempt to learn more about it the following experiments, mainly with healthy adult albino mice, were carried out. Two distinct and highly differentiated types of epithelial cells were made to stand out from one another by washing them while still alive with an ammoniacal silver solution. Only one of these is ciliated. The morphology and function of the other cell type—unciliated and much more conspicuous—are not well understood.

Material and Method

Each animal was anesthetized lightly with ether, the abdominal cavity opened, and the great vessels cut to exsanguinate the lungs. The diaphragm was carefully pinched up with fine forceps and nicked. With a hypodermic syringe inserted into the exposed trachea the now collapsed lungs were filled with silver solution to about the stage of full inspiration. After two minutes it was drained away, and the lungs were at once refilled with 10% formalin. The trachea was tied off, and the lungs and heart promptly removed and

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Contribution from the Department of Histological Research, Faculty of Medicine, the University of Western Ontario, London, Ont. A précis of this paper was kindly read by Prof. W. J. Hamilton of Charing Cross Hospital Medical School, the University of London, at the Summer Meeting of the Anatomical Society of Great Britain and Ireland on June 25th, 1948, in the University of Bristol.

[2] *Research Professor of Histology.*

placed in a bath of 10% formalin in which they remained for a day or more, standing exposed to daylight and at times direct sunlight. Frozen and paraffin sections were made, some of which were treated with direct sunlight and others with weak photographic developer. Many of the frozen sections were dried and flattened on the slide, and these were useful in looking over reaches of terminal bronchiolar epithelium. The paraffin sections were cut both thin and thick. All sections were examined clear, or after staining in different ways. The ammoniacal silver solution (Hodgman and Lange, 1930 (2)) was made up as follows:

Solution 1

Five grams of silver nitrate was dissolved in 300 cm.^{3} of distilled water and dilute *aqua ammoniae* was added until the precipitate formed was nearly but not entirely redissolved. It was then filtered, and water was added to make 500 cm.^{3}

Solution 2

One gram of silver nitrate was dissolved in a small quantity of water and poured into half a liter of boiling water. Rochelle salt (0.83 gm.) was dissolved in a small quantity of water and added to the boiling solution, which was then boiled for half an hour till a gray precipitate collected as a powder in the bottom of the flask. It was then filtered hot, and water was added to make 500 cm.^{3}

In using, equal parts of the two solutions were mixed. The separate solutions, kept in the dark, remain good for a month or two. Minor variations of composition were tried out, with approximately similar results.

Histological Observations

The low-power bird's-eye view (Fig. 1) of a typical cleared strip of silverized terminal bronchiole *t b* from a flattened frozen section is striking, being a mixture of sharply contrasting dark and light areas. Traced to the right the dark spots become less and less conspicuous, more scattered, and smaller, as we pass into the region of the alveolated duct at *a d*. A respiratory bronchiole in the usual sense is often difficult to discern in the mouse. As the intervening light areas are in a similar way traced to the right they come to occupy more and more of the presenting surface. At the left of the figure is part of the contour of a larger bronchiole *b*, and its surface also has a mottled appearance. The dark islands and tracts are the air surfaces of the silverized cells and the light ones are those of the relatively unsilverized cells. These are hereinafter called the dark and light cells, respectively. The dark cells are somewhat smaller in surface area than the others and are ciliated. The curious light or clear cells have protruding egg-shaped ends. They may be classified generally as columnar, though differing greatly from ordinary columnar epithelial cells.

They possess an individuality in high degree. The delicate cross striations in the middle of Fig. 1 are due to silver impregnation of the endothelium of the transverse bronchiolar pulmonary arteriole, *a*.

Fig. 2 is an oil immersion photomicrograph from the middle of a similar strip. The dark and light cells contrast strongly. The dark cells are more heavily outlined by silver deposits, and their surfaces are densely and fairly evenly covered with fine brown silverized granules of uniform size. The clear-cut cell contours are angulated, and the dark silver lines joining the projections are often incurved. The dark cells here occur singly or in groups of two, three, or more. The focal plane corresponds with the general level of the surfaces of the dark cells, which lie below the level of the apices of the domes of the light cells. No afterstaining was done, so that the nuclei and other details are not seen. The borders of the light cells are very imperfectly indicated by silverized granules. The contours are not acutely angulated, but rather tend to approach the circular form. The fact that they bulge laterally into the dark cells suggests that their protoplasm is of a firmer nature. Since their

EXPLANATION OF FIGURES

All figures are photomicrographs from the mouse lung except No. 14, which is from the lung of the golden hamster. All figures except two show only the effects of the supravital silverization, there having been no afterstaining. The exceptions are Nos. 12 and 13, which had no silver treatment, and were stained with iron haematoxylin. The technical details are given in the text.

Abbreviations:

a – *transverse bronchiolar pulmonary arteriole*
a d – *alveolated duct*
b – *bronchiole*
d c – *dark cell*
du c – *dust cell*
e b – *epithelial borderland*
j – *approximate point of junction of the paired terminal bronchioles shown*
t b – *terminal bronchiole*

Magnifications:

×*180* – *Figs. 1, 5, and 6.*
×*790* – *Figs. 7, 8, and 12.*
×*1800* – *Figs. 2, 3, 4, 9, 10, 11, 13, and 14.*

PLATE I

FIG. 1. *Flattened frozen section at 30 μ of a supravitally silverized lung, without afterstaining. It shows the surface pattern of dark and light cells in the epithelium of a terminal bronchiole. See text for description. S92-18Ms-3.*

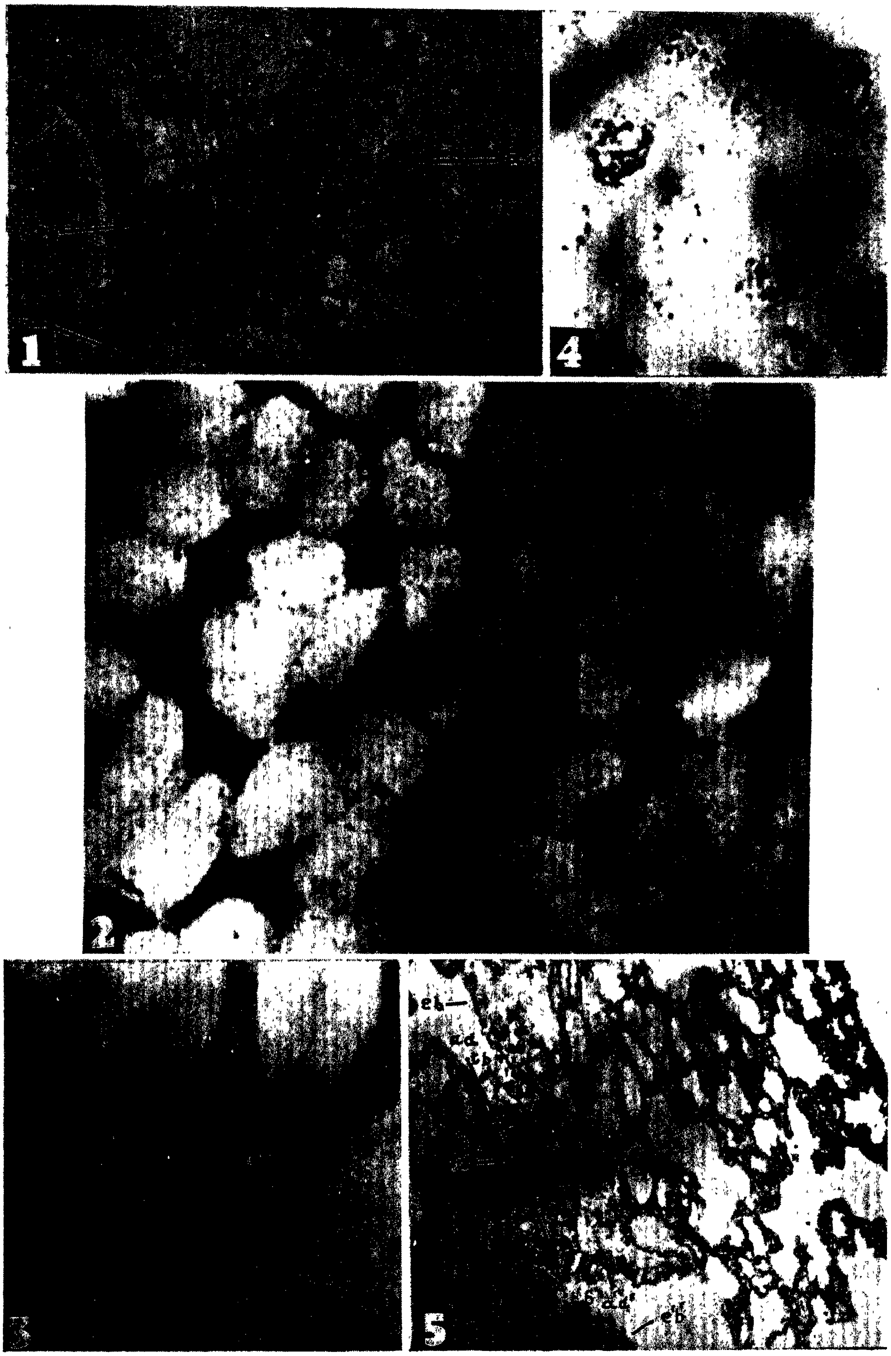
FIG. 2. *A small area from a field like that of Fig. 1, highly magnified. The finely granular faces of the dark cells and their sharply marked curved contours are outstanding features. S92-17Ms-1.*

FIG. 3. *The design and surface details of the dark and light cells of a terminal bronchiole in a flattened frozen section of 30 μ, unstained, at high magnification, are seen. S92-17Ms-1.*

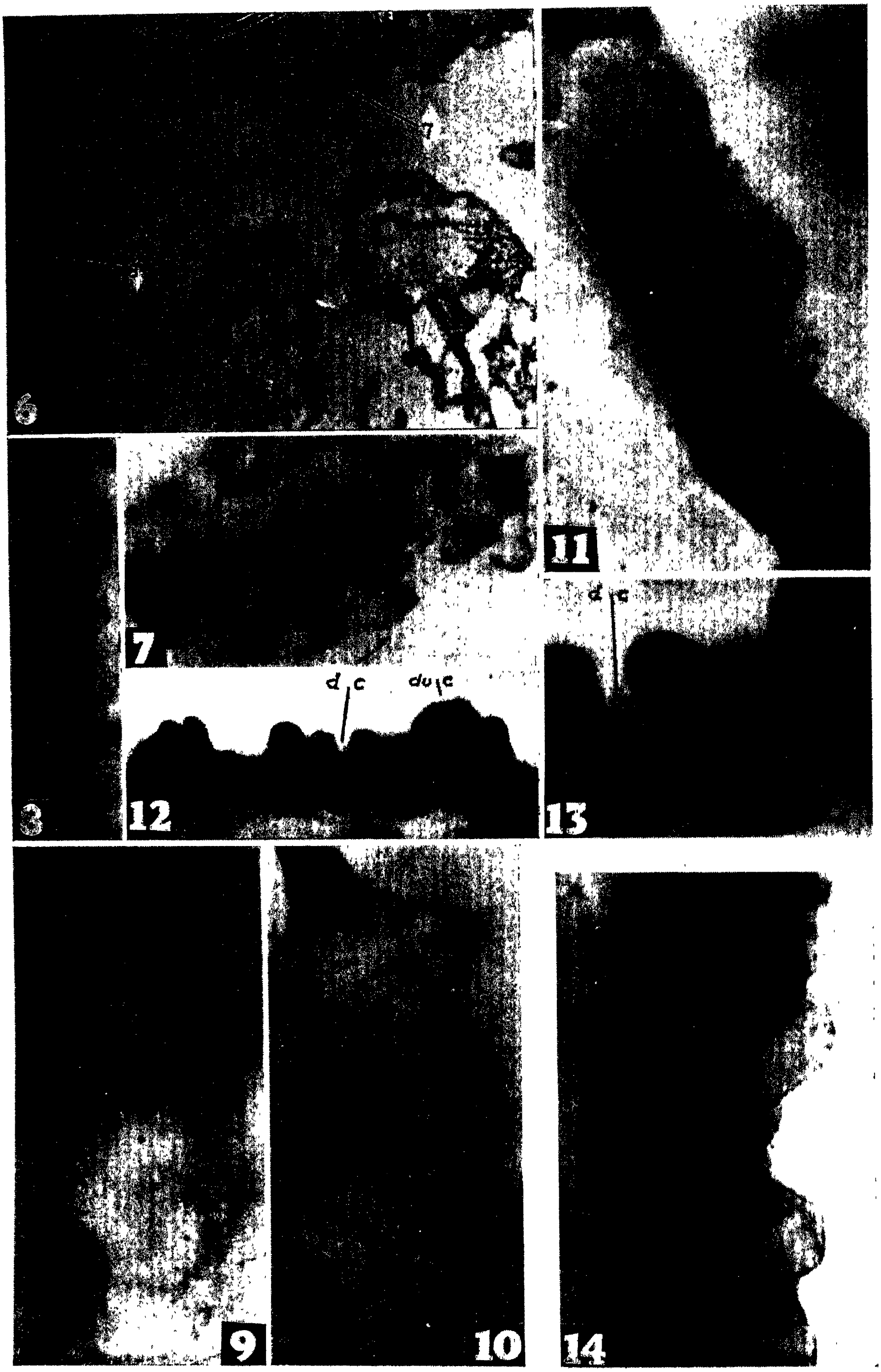
FIG. 4. *A high-level view of part of the field of Fig. 2 to give en face views of the granular plaques surmounting the domes of the light cells.*

FIG. 5. *The divaricated terminal bronchioles t b, t′ b′, are merged at j and present, in a 10 μ paraffin section, a strip of their epithelium in which the contrasting dark and light cell formations can be traced opposite to the regions of their respective alveolated ducts, a d and a′ d′. Attenuation of the dark cell pattern toward the epithelial borderland e b, e′ b′ is seen. S92-14Ms-8.*

PLATE I



FIGS. 1 TO 5.



FIGS. 6 TO 14.

tips, rising toward the eye of the observer, lie at a higher level than the focal plane of the photograph, they are not visible. By raising the objective, scattered fine dark granules are seen upon their egg-shaped domes; and surmounting the majority of these are discoid areas that contain more numerous granules. Fig. 3 is an oil immersion view of another surface of terminal bronchiolar epithelium. The boundaries between the light cells are marked by rows of black dots, and the surface stippling of the dark cells may be seen. The rounded plaques surmounting the domes of the light cells shown in Fig. 2, appear in an oil immersion surface view in Fig. 4. In some of these a fairly complete ring of small and intensely dark granules is on or just within the periphery. It is often difficult to distinguish the boundary of the disk from the contour of the underlying dome. The significance of these plaques is unknown.

In Fig. 5 we have a view at low power of part of the confluential walls of two terminal bronchioles, *t b*, *t′ b′*, pointing in opposite directions to their respective alveolated duct systems, *a d* and *a′ d′*. The approximate junctional point *j* is presumably opposite the supply bronchiole, which is not included in

PLATE II

(For general explanation of figures see p. 52)

FIG. 6. *Profile views of dark and light cells in a bronchiole b and a terminal bronchiole t b are seen in a 10μ cleared paraffin section. At 7 and 8 are indicated small lengths of silverized epithelium, which are more highly magnified in Figs. 7 and 8, respectively. S92-14Ms-8.*

FIG. 7. *The dark cells in this terminal bronchiole are short and often pyramidal in shape. Their dense cuticular parts are lowermost. Between them are the light cells, whose protruding ends are imperfectly outlined in fine silvered granules.*

FIG. 8. *Dark and light cells from the wall of bronchiole b, Fig. 6, are seen. The dark cells tend to be in larger groups, and the light cells lower, than in the terminal bronchiole, Fig. 7.*

FIG. 9. *A highly magnified view of silverized terminal bronchiole epithelium is seen in this 10μ paraffin section. The middle cell, a truncated pyramid, has densely silvered edges. Its contracted apex is dark golden brown in color. S92-14Ms-8.*

FIG. 10. *The dark cells of Fig. 9 are seen at a lower focal level. The narrow cuticular air face of the middle cell is distinct at the bottom of a funnel-shaped depression, hedged in by the projecting ends of the light cells.*

FIG. 11. *A short length of epithelium from a bronchiole larger than that of Fig. 6, b is seen in this 10μ paraffin section. The sharply outlined, relatively high dark cells have flaring bases and bear tufts of cilia on their narrow cuticular surfaces. The spheroidal light cells press laterally into the dark cells, and have low domes projecting into the lumen. S92-14Ms-8.*

FIG. 12. *In this unsilverized material, fixed in Bouin's fluid, cut at 10μ from paraffin and stained with iron haematoxylin and eosin, the dark cells, low and inconspicuous, might easily be overlooked. One of them , d c, shows a sharp apex. The rounded projections of the light cells here stain densely. S92-23Ms-2P-2.*

FIG. 13. *This is a high magnification of a part of Fig. 12.*

FIG. 14. *In this section of silverized lung from the golden hamster the fundamental characteristics of silver impregnation, and of the morphology of the dark and light cells, are the same as those of the mouse. In this terminal bronchiolar epithelium the dark cells are lower than are the light cells, and their narrow cuticular areas, bearing cilia, are flanked by the bulging ends of the light cells. S92-15H-1, paraffin section, 25μ.*

this section. The pattern of dark and light areas is typical. As in Fig. 1, the dark cell faces become less conspicuous toward the alveolated ducts. Some side views of dark cells are imperfectly visible in the epithelial borderland *e b*, *e′ b′*. Low-power profile views of bronchiolar and terminal bronchiolar epithelium after supravital silverization are given in Fig. 6. To the left is a bronchiole *b* leading into a terminal bronchiole *t b* cut lengthwise. The dark and light areas representing the two types of cell of the epithelial coat are plainly seen. Owing to unevennesses in the epithelial layer, the cells are not always cut so as to present their long axes exactly in the plane of the section. In the wall of the bronchiole *b* the dark cells are larger and more regular than they are in the terminal bronchiole. The numerals 7 and 8 indicate the areas of epithelium shown in Figs. 7 and 8, respectively, at higher magnification. The dark cells seen in Fig. 7 appear short and tend to be triangular in outline. The air surfaces face downward. The air face of each dark cell is formed by a mass of somber brown silverized material. The cell outlines are indicated by layers of fine black granules. The protuberances of the light cells, extending downward below the levels of the apices of the contiguous dark cells, are faintly outlined in sparse fine dark granules. The air surface in Fig. 8 faces the right: here the dark cells have a more regular outline and occur in larger groups. Their exposed faces, strongly marked in dark brown silver deposit, are relatively broader and bear granules whose linear arrangement suggests that it has been determined by the presence of cilia. The boundaries of these faces are, as before, outlined by numerous small granules. The light cells, unciliated, do not project quite as prominently as in the terminal bronchiolar epithelium of Fig. 7.

The typical pyramidal shape of a dark cell situated near the margin of a terminal bronchiole is seen in the oil immersion view from another region of this section (Fig. 9). The other dark cells above and below in this field are not axially in focus. The very narrow apex appears dark brown and is almost buried between the towering ends of the contiguous clear cells. The granules marking the sides and broad base are probably a precipitate of silver, and are black, rather than brown. The angle lines of the sides and base are very strongly pencilled. These cells appear in Fig. 10 at a deeper focal level. For comparison with the foregoing we have in the oil immersion view of Fig. 11 the dark and light cells from a larger bronchiole of the same section. The dark cells are here somewhat higher and bear granular formations suggesting definite tufts of cilia. The walls of the dark cells are typically incurved, presumably from contact with the light cells, and their bases are often flaring. As before, their outlines are marked by fine black granules of silver. In the conspicuous golden brown cuticle an inner and an outer thin layer of darker material, separated by a thin layer of lighter substance, can be made out in the section, but not clearly in the figure. These darker layers can be resolved into brown granules in vertical pairs, apparently basally related to the cilia.

The light cells are here more nearly circular in outline, and do not show such extreme prominences, and the air surfaces are sprinkled with golden brown granules and appear as faint lines in the photomicrograph.

We now turn to the appearance of the terminal bronchiolar epithelium of the mouse after one of the more usual methods of preparation (Fig. 12). This lung was not silverized, but was fixed in the same way as the others by injection of Bouin's fluid (Masson, 1929 (11)). The dark projections with rounded ends represent the light cells, and among them, suggesting gaps in a row of teeth, are low regions filled in by the dark cells, which now appear of lighter tone. One of these *d c* is distinctly of triangular contour, with an almost needlelike apex, which on higher magnification suggests the tip of a wetted and compacted camel's hair brush—but discrete cilia cannot be made out. Riding upon the marginal light cells are two dust cells *du c*. It seems probable that these are on their way from the alveoli to the region of the pharynx. Part of the epithelium shown in Fig. 12 appears under the oil immersion lens as in Fig. 13. The sharp point of the triangular dark cell *d c* might be called a stereocilium; but there is nothing here to decide the question of whether or not it is motile.

Fig. 14 is an oil immersion photomicrograph of the epithelium of a terminal bronchiole of the golden hamster. It is appended as evidence that the specializations already noted in the dark and light cells are not peculiar to the mouse. The short heavily silverized dark cells and the relatively opulent light cells are as sharply contrasted as those of the mouse.

Control lung sections, cleared without staining, from animals whose fresh lungs were fixed intrabronchially without silverization, showed no dark granules like those herein described for the silverized material.

Discussion

It is not intended here to deal with the epithelium of the terminal bronchioles in a broad way, but to highlight the peculiar and striking surface phenomenon attained by supravital washing with ammoniacal silver solution. Apparently the two types of cells of the terminal bronchioles have been known for almost (if not more than) 70 years; for Miller (1932 (12)) wrote (p. 134): "As stated by Kölliker (1881 [3]), ciliated epithelium extends beyond the point where goblet cells disappear; it does not, however, form a complete layer; for, interspersed with the ciliated cells, there are numerous non-ciliated cells." This statement Miller (1947 (13, p. 19)) repeated. Speaking of bronchiolar epithelium Miller (1932 (12, p. 137)) wrote: "In a longitudinal section of a bronchiolus which is dividing into bronchioli respiratorii a still further transformation of the epithelium can be followed. The cilia-bearing cells gradually disappear and are replaced by non-ciliated cuboidal epithelium which can be best seen in transverse sections of bronchioli respiratorii which have but few alveoli along their walls." Miller (1932 (12, p. 134)) also wrote: "Moreover, the type of the cilia-bearing cells changes from a columnar to a cuboidal epithelium, which is the predominating type of epithelium in bronchioli between 0.3 mm. and 0.4 mm. in diameter," and repeated this statement (1947 (13, p. 19)). He did not, however, describe any pyramidal cells with contracted apices such as I show in my Fig. 9. He mentioned the "finely granular ends" of the ciliated cells (Miller, 1932 (12, p. 135)).

I feel that the supravital silverization method that I have herein described makes visible my "dark cells" (which fundamentally are ciliated epithelial cells, although the cilia seem to become rudimentary, and even sometimes invisible with ordinary means) farther down the air tract than is possible by usual methods. Although the cuticular plates of the pyramidal cells in the marginal region of the terminal bronchiolar epithelium become very narrow, and separate cilia arising from them have not always been discernible, yet the presence of cilia on all of them has not been ruled out, and the occurrence of an outward-bound fluid current set up by them here is possible. Lacking this the dust cells must make their way toward the pharynx by their amoeboid efforts alone. This air channel, rather than the lymphatic vessels of the lung, is regarded as their normal highway in leaving the lung (Macklin, 1938 (7)). The paired granules seen in the cuticle in lateral views are doubtless the "basal corpuscles" related to the roots of the cilia, referred to by histologists. It is possible that the dark cells are homologous distally with the epicytes (septal cells, niche cells, alveolar granular cells) of the alveolar walls (Macklin, 1938 (6), 1946 (8)). Both become superficially marked by a beautiful golden brown granulation after supravital washing with silver solution.

The light cells, with their free processes, are prominent objects in the sections. These projections suggest villi, so that the term "villus cells" is not inappropriate (Macklin, 1948 (10)). They are often shorter at the distal edge of the epithelium than a short distance proximal to this. If a fixative having a shrinking action is used, the sides of the processes are approximated, and then the presenting air surface looks fairly smooth, or at most gently scalloped. These villus processes are best seen by using the intrabronchial fixation method, with very low pressure. In ordinary immersion-fixed material, such as that used by pathologists, the processes are crowded together and hence seen with difficulty if at all. There may be an homology distally between the light cells and the so-called "bare areas" (Macklin, 1937 (5); 1938 (6)) of the alveolar walls, by some workers thought to be occupied by thin squames of epithelial origin, invisible in ordinary histological preparations. The possibility of dust and foam cells of the alveoli originating from the bronchiolar cells has been discussed (Macklin, 1947 (9)). The specialized nature of the mitochondria in the light cells (Macklin, 1948 (10)) is being presented in a separate paper.

From the foregoing exposition it is clear that the usual descriptions of the terminal bronchiolar epithelium are inadequate for the mouse. Most of the histological texts (as, for instance, Miller, 1932 (12) and Heiss, 1936 (1)) present the ciliated cells as here "disappearing" and being replaced by cuboidal cells, or as losing their cilia and becoming cuboidal. It would seem that the light cells herein described represent many at least of the "cuboidal epithelial cells" of these descriptions, though there is nothing to prove that they have ever borne cilia. It is possible that they may be homologous proximally with the goblet cells found in larger bronchioles. It has been herein noted that the findings in the golden hamster by this silverwash method are like those in the mouse. Sections from Bouin-fixed material, stained by common methods, have been examined in nine other mammals—rat, baboon, dog, goat, guinea-pig, monkey, rabbit, cat, man—and representatives of what are taken to be the two types of cells were found in the epithelium of the finest bronchioles. From these results it is felt that the pattern, morphology, and function of the dark and light cells are here basically comparable throughout this class, though there are notable species differences of detail.

Acknowledgments

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ANALYSIS OF THE ESSENTIAL STRUCTURAL FEATURES OF DDT BY A STUDY OF THE TOXICITY OF CLOSELY RELATED COMPOUNDS TO ROACHES AND TO HOUSEFLIES[1]

By Jean P. Picard[2] and C. W. Kearns[3]

Abstract

Three series of compounds have been prepared and compared for the insecticidal activities against German roaches and houseflies. The substituted 2,2-diphenyltrichloroethanes and substituted benzohydrols have been found to have comparable activities on roaches. The substituted 2,2-diphenyltrichloroethanes alone had insecticidal properties against houseflies. The benzophenone derivatives were ineffective against both groups of insects.

I. Introduction

Since the discovery of the outstanding insecticidal properties of DDT (1-trichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane) in 1939, a considerable amount of work has been published on the relationship between the chemical structure and the activity of the DDT molecule as an insecticide.

Lauger *et al.* (1944 (6)) was probably the first to point out the possible relation between the chemical structure and the toxicity of DDT. He postulates that the extreme poisoning effect of DDT is due to the basic toxicity of *p*-bridged 4,4′-dichlorodiphenyl structure, which probably acts by an enzyme-blocking mechanism while the chloroform residue, he suggests, acts like a lipophylic group, which causes the molecule to have an affinity for the nerve lipoids.

On the other hand Fleck and Haller (1944 (4)), from a study of the ease with which hydrochloric acid could be released catalytically from the DDT molecule, have suggested that the insecticidal properties of DDT might be due to the catalytic decomposition by a naturally occurring catalyst. The same year Martin and Wain (1944 (7)) have reported that 4,4′-dichlorobenzophenone and 4,4′-diphenylacetic acid were almost inactive as contact insecticides and stomach poisons indicating that the $CHCCl_3$ group is associated with the toxic properties of the molecule. They claim that the hypothesis of the intracellular decomposition of DDT is preferred. The toxicity of the latter is ascribed to the hydrochloric acid simultaneously produced by either elimination or reduction. According to these workers, the insecticidal properties of a compound to which this hypothesis is applicable would depend on the ease with which hydrochloric acid is produced, provided that the compound is stable enough to survive translocation to its site of action.

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In view of these facts we thought that some information could be obtained about this subject by first preparing analogues of the DDT molecule varying the para-substituent and measuring quantitatively their relative potency, and then comparing measurements with similar molecules known to be incapable of dehydrohalogenation.

Analogues of the DDT molecule were synthesized and compared to closely related compounds in which the $CHCl_3$ of the former was replaced by C=O and by CHOH. This last group of compounds has not been previously investigated for insecticidal properties.

The preparation of these compounds and the evaluation of their relative potency are the object of the present study.

II. Preparation of Compounds

Three series of compounds were prepared and evaluated on insects: Series 1, substituted 2,2-diphenyltrichloroethanes; Series 2, substituted benzophenones; Series 3, substituted benzohydrols.

1. *Substituted 2,2-Diphenyltrichloroethanes*

The syntheses of the DDT analogues was carried out by the Baeyer reaction (1827 (1)) between chloral and aryl derivatives using for the condensing agent a 1 to 1 mixture of concentrated sulphuric acid and fuming sulphuric containing as much as 20 to 30% of sulphur trioxide.

The condensation of chloral with monohalogenated benzene derivatives proceeds readily in presence of sulphuric acid mixture but in the case of the alkyl and alkoxyl benzene derivatives, owing to their high reactivity, a mixture of acetic acid and concentrated sulphuric acid was used instead of the usual condensing medium.

2. *Substituted Benzophenones*

The preparation of the substituted benzophenones was accomplished through the extension of a method used by Boesken (1905 (2)) for the preparation of benzophenone dichloride by the action of carbon tetrachloride on benzene in the presence of anhydrous aluminium chloride. When these compounds were subsequently hydrolyzed by steam, their corresponding ketones were obtained.

This method of preparation for symmetrical halogen and alkyl substituted ketones seemed more efficient than the process of condensing substituted benzoyl chloride with halogen or alkyl derivatives of benzene.

The condensation of monohalogenated and monoalkylated derivatives of benzene proceeded at room temperature without the formation of resinous by-products.

Attempts to prepare the 4,4′-dimethoxyl by the same method were unsuccessful.

3. *Substituted Benzohydrols*

The symmetrical aromatic ketones previously prepared were reduced to the corresponding secondary alcohol in almost quantitative yield by the use of aluminium isopropylate.

Since the proposal of this method by Meerwein and Schmidt (1925 (8)) and by Ponndorf (1926 (9)) for the reduction of a carbonyl group to the corresponding alcohol an extensive amount of work with this method has been reported, but as yet nothing has been published on the application of this method to the symmetrical substituted aromatic ketones.

The aromatic ketones can also be reduced to the corresponding secondary alcohol by alcoholic alkali and zinc dust. The only drawback to this method is the formation of pinacol, which, according to Cohen (1919 (3)), is the first step in the reduction.

III. Methods of Testing

1. *Using Roaches*

A quantitative measurement of the relative potency of each compound prepared was accomplished by determining the amount of the compound, in milligrams per 929 cm.^{2}* required to kill 50% adult male German roaches, *Blatella germanica* L., continuously exposed for 96 hr. to surfaces coated with the compound. This amount was called the LD_{50} (lethal dose to kill 50% for 96 hr. exposure).

The actual testing of the material was carried out by coating pint jars with acetone solutions containing various concentrations of the compound to be tested. The total surface area of a jar was found to be 929 cm.^{2}* Knowing the surface to be coated, the concentration of the solution to be used, and the number of mgm. per 929 cm.^{2} desired, the proper volume of the acetone solution was added to the jar by means of a 0.05 cc. graduated burette. The jars were uniformly coated by rotating them by hand until complete evaporation of the acetone solution. Ten male German roaches, after anesthesia with carbon dioxide, were confined in each jar and a minimum of 10 jars were prepared for each concentration to be tested.

2. *Using Houseflies*

The compounds prepared in this study have also been evaluated as contact insecticidal sprays on the housefly, *Musca domestica* L., by a wind tunnel method.

The apparatus used consists of a small wind tunnel, described by Roan and Kearns (1948 (10)). The air stream and the atomized spray droplets are pulled from one end to the other by means of an electrically driven blower. The test cage is placed in the apparatus in such a manner that it forms a section of the wind tunnel through which the spray flows. The time of exposure is limited by the velocity of the air stream and by the air pressure used to operate the atomizer.

* *929 cm.^{2} = 1 sq. ft.*

The flies, after being sprayed, are transferred to recovery cages. These cages consist of one quart cardboard liquid containers with the cardboard ends removed. The end and covers are replaced by 16 mesh galvanized screen of the same diameter. The container is lined with a section of kraft wrapping paper, which may be removed and discarded after use. The screen ends and test-cages are decontaminated by standing over night in a solution of alcoholic potassium hydroxide.

The material to be tested is dissolved to the proper concentration in a highly refined kerosene ("Deo-base"). The volume of the spray solution is 0.5 cc. Each test was repeated five times. Checks run with 0.7 cc. of "Deo-base" alone gave less than 5% mortality.

IV. Results

The replacement of $CHCCl_3$ by CHOH in the 4,4′-disubstituted analogues of DDT, in which both substituents were fluorine, chlorine, bromine, methyl, and methoxyl, appear to be as toxic to male German roaches as the corresponding DDT analogue.

The same is true of the analogue without substituent in either ring although a considerable decrease in activity is apparent.

The LD_{50} for each compound was determined by plotting on logarithmic-probability paper, the percentage mortality at various concentrations. These results are shown in Tables I and II.

The benzophenone derivatives have been tested at 10 times the concentration required by the corresponding hydrol to kill 50% of the roaches, and even at that high concentration none of them show any sign of activity.

The results obtained for these same compounds tested as insecticidal sprays against houseflies are illustrated in Table III. In this experiment, if a compound did not show any activity at a concentration of 5 mgm. per cc. or 0.5%, it was not investigated further. This was the case with benzophenone and benzohydrol derivatives, which were found to be ineffective at a concentration of 5 mgm. per cc.

The 4,4′-dimethoxyl compound, although slightly less effective than DDT, has a considerable "knockdown" effect.

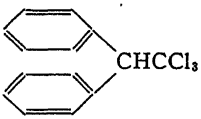
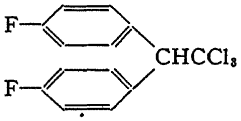
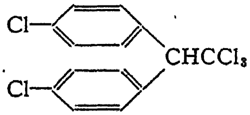
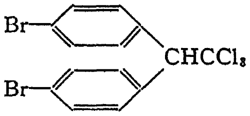
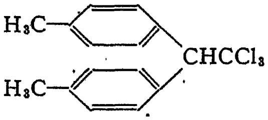
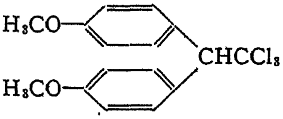
V. Discussion

In attempts to explain the essential structural features of DDT (1-trichloro-2,2-*bis*(*p*-chlorophenyl)-trichloroethane), several theories have been proposed.

The necessity of $CHCCl_3$ group as lipophylic agent postulated by Lauger *et al.* (1944 (6)), the possibility of dehydrohalogenation at the site of the action proposed by Martin and Wain (1944 (7)), the ease with which the DDT molecule could lose hydrogen chloride in presence of a naturally occurring catalyst, and the spatial relationship of the different parts of the molecule considered by Gunther and Metcalf (1946 (5)) are among the most important theories suggested for the mechanism of action of DDT-type compound.

TABLE I

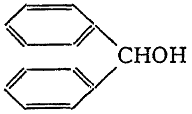
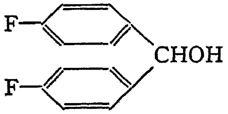
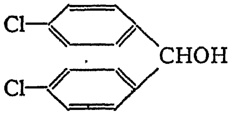
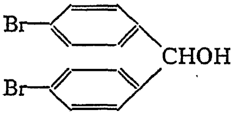
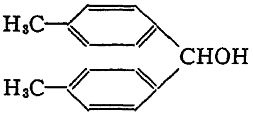
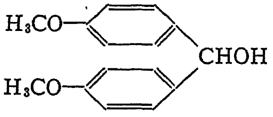
PERCENTAGE MORTALITY OF MALE GERMAN ROACHES EXPOSED TO SURFACES TREATED WITH VARIOUS AMOUNTS OF SUBSTITUTED 2,2-DIPHENYLTRICHLOROETHANE

Compound	Mgm./929 cm.^{2}	% Mortality at 96 hr. exposure	LD_{50}*
 1-Trichloro-2,2-diphenylethane	5 10 20 40 50	32 42 57 64 70	15
 1-Trichloro-2,2-*bis*-(*p*-fluorophenyl)-ethane	0.01 0.05 0.1 0.4 1	42 56 65 77 82	0.025
 1-Trichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane	0.2 0.3 0.5 1. 1.5	4 11 20 43 60	1.15
 1-Trichloro-2,2-*bis*-(*p*-bromophenyl)-ethane	0.5 1 3 4 10	11 18 42 58 70	3.4
 1-Trichloro-2,2-*bis*-(*p*-methylphenyl)-ethane	2 5 10 15 20	36 50 65 70 77	4.6
 1-Trichloro-2,2-*bis*-(*p*-methoxyphenyl)-ethane	0.5 1 2 3 4	6 14 36 49 62	3.2

* *LD_{50} (lethal dose for 50% mortality) in mgm./929 cm.^{2} after 96 hr. exposure.*

TABLE II

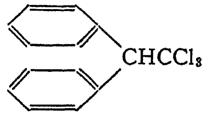
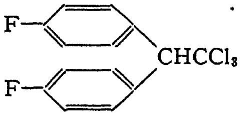
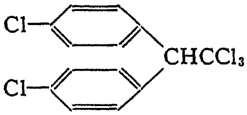
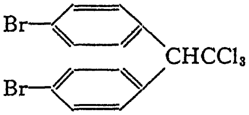
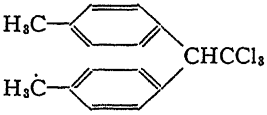
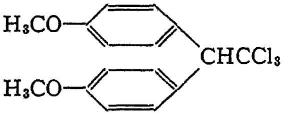
PERCENTAGE MORTALITY OF MALE GERMAN ROACHES EXPOSED TO SURFACES TREATED WITH VARIOUS AMOUNTS OF SUBSTITUTED BENZOHYDROL

Compound	Mgm./929 cm.^{2}	% Mortality at 96 hr. exposure	LD_{50}*
 Benzohydrol	5 10 20 40 60	34 43 54 63 72	15
 4,4′-Difluorobenzohydrol	0.01 0.05 0.1 0.5 1	40 52 60 66 72	0.03
 4,4′-Dichlorobenzohydrol	0.3 0.5 1 1.5 2	9 20 36 55 64	1.3
 4,4′-Dibromobenzohydrol	0.5 1 3 5 10	10 18 38 54 86	4
 4,4′-Dimethylbenzohydrol	1 2 5 10 20	24 35 53 67 80	4.2
 4,4′-Dimethoxybenzohydrol	0.5 1 2 3 5	4 15 31 50 61	3.5

*LD_{50} *(lethal dose for 50% mortality) in mgm./929 cm.^{2} after 96 hr. exposure.*

TABLE III

PERCENTAGE MORTALITY, AFTER 24 HR., FOR HOUSEFLIES SPRAYED WITH 0.5 CC. OF VARIOUS CONCENTRATIONS OF SUBSTITUTES 2,2-DIPHENYLTRICHLOROETHANE

Compound	Mgm./cc.	% Mortality after 24 hr.	LD_{50}*
 1-Trichloro-2,2-diphenylethane	5	0	
 1-Trichloro-2,2-*bis*-(*p*-fluorophenyl)-ethane	2.5 2 1 0.5	95 86 17 50	0.55
 1-Trichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane	0.75 0.5 0.25 0.1	94 85 70 41	0.17
 1-Trichloro-2,2-*bis*-(*p*-bromophenyl)-ethane	1 0.75 0.5 0.25	95 82 70 50	0.28
 1-Trichloro-2,2-*bis*-(*p*-methylphenyl)-ethane	5 4 2.5 1.5	94 84 60 22	2.20
 1-Trichloro-2,2-*bis*-(*p*-methoxyphenyl)-ethane	2 1 0.75 0.5	94 70 40 29	0.80

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* *LD_{50} (lethal dose for 50% mortality) in mgm./cc. after 24 hr.*

The high activity of the hydrol corresponding to the DDT analogues, leads one to believe that the lipoid soluble group $CHCCl_3$ is not necessarily required in order for the DDT-type compound to be active and that a molecule of this type, deprived of its ability of losing hydrogen chloride, could still act as an insecticide.

In regard to insecticidal activities, the compound must be substituted in the 4,4′-positions, as demonstrated by the low activity of unsubstituted analogues, and the substituents must be of limited size. This point is well elucidated by the low LD_{50}'s found for the 4,4′-dimethoxyl and 4,4′-dimethyl compounds as compared with their next higher homologues. The difference is at least 10-fold.

The fact that toxicity in the DDT analogues vanished with the increase in the *p*-alkyl or *p*-alkoxyl substituents, indicates that enhanced paraffinic character is not beneficial even if it favors oil-solubility of the product.

The variation in toxicity within the 2,2-disubstituted ethanes, the difference in activity with the benzohydrol derivatives, and the inactivity of the benzophenones, when tested on two different groups of insects, suggest that the electronegative character of the substituents and the lipophylic action of the $CHCCl_3$ group cannot alone account for the significant action of this general type of structure, but rather the whole shape of the molecule must be considered. A mere mechanical view of tissue penetration by any contact poison leads to this consideration. This is also supported by the fact that molecules of other than a certain size were found to possess low activity. The nonrequirement for the molecule to bear a $CHCCl_3$ group also supports this view.

These findings tend to demonstrate the importance of exploring the potency of related compounds on more than one species of insects before postulating the relationship between the chemical structure and toxicity. This point is strikingly illustrated by the high activity of the hydrols on the German roach and their relative inactivity when applied to housefly.

VI. Acknowledgment

The authors are indebted to the Department of Lands and Forests of Quebec for the generous financial support of this project. Thanks are also due to Miss Amy Kell and Mr. H. K. Miller for aid in the experimental work.

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THE FUR RESOURCES OF CHAPLEAU DISTRICT, ONTARIO[1]

BY RANDOLPH L. PETERSON[2] AND VINCENT CRICHTON[3]

Abstract

Records of the area trapped and of legally caught furs in the Chapleau district, Ontario, have been made for the seasons 1941-42 to 1946-47. From these records the area required to produce one harvested animal has been computed for most species, which should be of considerable value to persons concerned with fur production in Canada. These figures should also be useful to students of animal populations since they take into account, at least in part (specific areas actually trapped), the variation in trapping pressure that cannot be reflected in figures based on total numbers taken from large areas. This six year period seems sufficient to indicate significant trends in populations, which in certain cases might be predicted by study of past trends of province-wide returns.

During the six year period the average return value for each square mile trapped was $16.90, which netted each trapper using one township an average of $607.00 each year. The average total annual fur resources amounted to $74,000.00.

The distribution of the catch of marten and fisher in the district suggests that the Chapleau Crown Game Preserve may be influencing the catch of these two species in nearby territories.

Introduction

The value of wild caught furs of Canada's 31 million dollar fur resources amounted to approximately eighteen and one-half million dollars in the season 1944-45 (1). Although total fur return figures are available for the various Provinces and for the Dominion as a whole, these figures give no index to specific fur production from small comparable areas under various levels of trapping pressure. It appears desirable to know what furs have been taken from a specific area and what relation the trapping pressure has to the size of the area necessary to produce one trapped animal. Even within a district the size of Chapleau, the actual area trapped varies from year to year. In other words, how large an area is necessary to produce one harvested animal unit for each of the various species being taken from any given district?

The Chapleau Forest District (Fig. 1) is an administrative unit of the Ontario Department of Lands and Forests (formerly under the Game and Fisheries Department and later amalgamated with the Department of Lands and Forests as the Fish and Wildlife Division). Coauthor Crichton, an officer of the Fish and Wildlife Division of the Department, has the responsibility of administering the fish and wildlife resources of this district. His primary responsibility is law enforcement, with some biological investigations as well as administration. The area has been divided into trapping grounds and each trapper assigned to a definite trapping area. For the most part each trapping ground is equivalent to one township (36 square miles), although some trappers have been allotted one and one-half or two townships. These grounds are usually held year after year by the same trapper (Fig. 2). Each trapper is required to report the exact number of furs of each species taken. (The illegal trapping presents a factor that cannot be properly evaluated here.) With the exception of beaver, there was no restriction on the number of pelts that could be taken. Over the period here discussed, the take of beaver was regulated by the Provincial Government to insure sustained annual yield and was limited roughly to an average of 10 beaver per trapping ground. Beginning with the season 1941-42, a careful record of each pelt legally taken from each ground has been made under the supervision of coauthor Crichton. The annual catch from each individual trapping ground has been compiled and these data computed for the entire area trapped in the Chapleau district, with the exception of the southernmost 14 townships, which were added to the district beginning with the 1946-47 season. For purposes of this paper these townships are considered as not being trapped and are not included with the area trapped except in the 1946-47 season. Thus, prior to 1946-47, the actual production of the entire district was greater than the figures here given but the size of the unit area required to produce each pelt was not affected.

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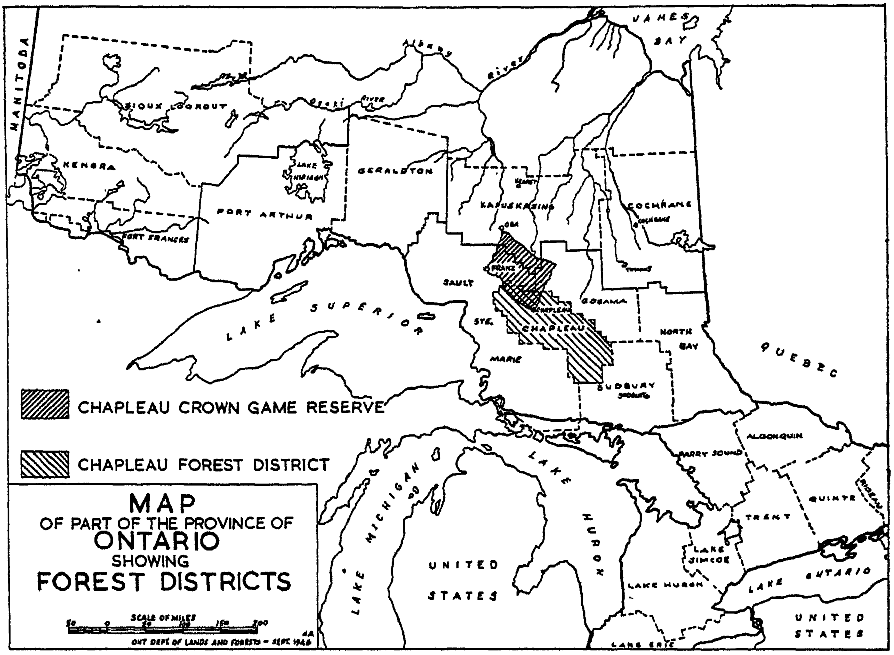

FIG. 1. *Map of part of the Province of Ontario showing the Chapleau Forest District and the Chapleau Crown Game Preserve.*

The relative intensity of trapping pressure within the various trapping grounds cannot be properly evaluated or compensated for in computing the size area necessary to produce one animal unit because of differences in trapping techniques, number of traps utilized, general skill and ability of trappers, intensity and length of active trapping, amount of selective trapping, and various other uncontrollable factors. Further, it should be remembered that several types of habitat are found throughout the district, thus the productivity for the various species necessarily varies even within small localities. A notable example is the habitat of the muskrat; its aquatic habitat occurs only in certain townships and in those is often restricted to a small percentage of the area. On the other hand, most of these trapping grounds are trapped annually by the same trappers, which should tend to create fairly equal harvesting pressure except in cases where changes in relative value of various species and other factors have tended to cause selective trapping for the more valuable types. Even so, it still seems highly desirable to know what the average productivity of a given tract of northern Ontario land might reasonably be expected to be under more or less "normal" trapping pressure.

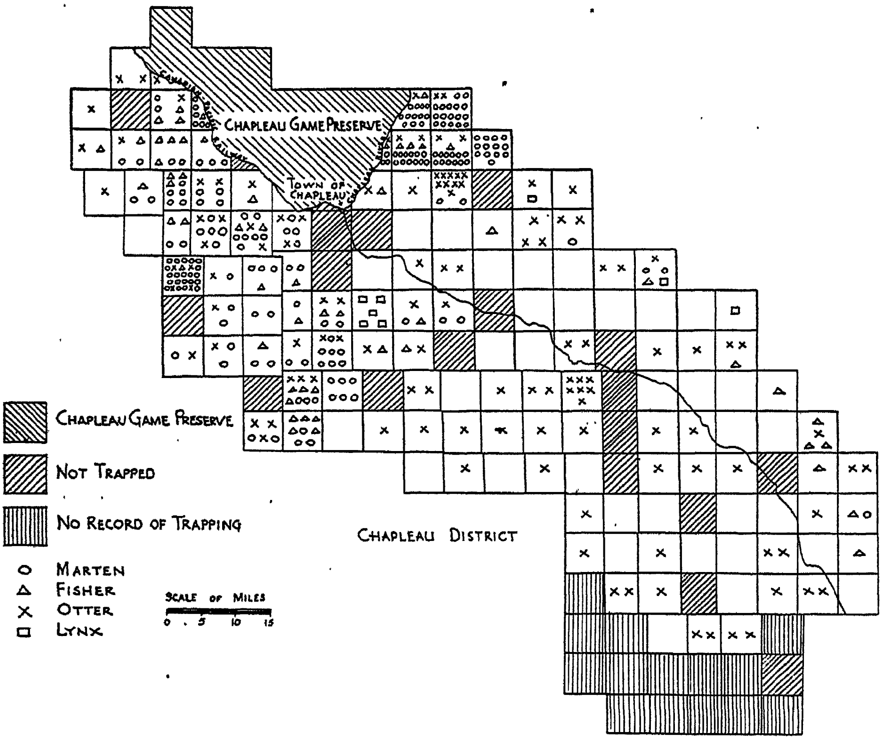

FIG. 2. *Map of the Chapleau Forest District showing area trapped in 1945-46 and distribution of catch of marten, fisher, otter, and lynx for that season.*

Although comparable figures for fur production of other districts within Ontario are not available, the Chapleau district probably rates well above average in fur production for areas of comparable size throughout the Province.

We are grateful to Prof. J. R. Dymond, Director, Royal Ontario Museum of Zoology; Dr. C. H. D. Clarke, Wildlife Supervisor, Ontario Department of Lands and Forests; Dr. Walter P. Taylor, Senior Biologist, United States Fish and Wildlife Service; and others for their co-operation, suggestions, and reading of the manuscript.

Description of Area

The Chapleau Forest District, lying east of Lake Superior, averages roughly 60 miles wide and extends some 190 miles northwest and southeast along the Canadian Pacific Railway, which bisects the area lengthwise (Fig. 1). The total area is approximately 6384 square miles, about 600 square miles of which lies within the Chapleau Game Preserve, which extends into the northern portion of the forest district, leaving roughly 5780 square miles of trapping grounds.

The topography is made up of many lakes and streams winding through low rolling ridges with considerable outcroppings of rocks, especially along and south of the height of land that extends across the northern portion of the district. The geologic formations are chiefly Precambrian, with most of the district lying on the Canadian Shield. The town of Chapleau, situated just north of the height of land, has an elevation of 1412 ft. above sea level. The district is divided into three watersheds. The drainage of the northern portion flows into the Moose River drainage to James Bay. The northwestern portion lies in the Lake Superior drainage, while the remaining southern portion is situated in the Lake Huron drainage.

The entire Chapleau district would fall within the Canadian Life Zone of Merriam, Bailey, Nelson, and Preble (13) but certainly there are marked differences between the flora of the northern and southern portions of the district. Several species of trees reach their northern limits in the Chapleau vicinity. These include white pine, hemlock, yellow birch, red maple, sugar maple, striped maple, basswood, red oak, black cherry, beech, and others. This would seem to indicate a valid separation of biotic areas within this area as proposed by Halliday (8) and Dice (7). Jack pine is the only pine found to any extent in the far northern portion of Chapleau district, while both white and red pine occur with it in the southern portions. The common species found throughout the district include black spruce, white spruce, white birch, white cedar, quaking aspen, balsam poplar, large-toothed aspen, cherries, Juneberry, and several smaller shrubs such as alders, dogwood, and willows. The southeastern half of the district has been extensively logged and burned, resulting in large areas of second growth poplar and white birch on rather thin soils.

For a list of the summer birds for the Chapleau area see Baillie and Hope (2).

History of the Area

During the early exploration and trading in northern Canada the Hudson's Bay Company restricted most of its posts and settlements to the coastal areas. One of their earliest inland posts was a temporary one called Brunswick House built about 150 miles north of the Chapleau area on the Missanabie River in 1776 (10). According to Voorhis (14) this post was abandoned in 1790 and a New Brunswick House was substituted. The latter was built in 1778 at the north end of Brunswick Lake, which is now a part of the Chapleau game preserve. Later several other Hudson's Bay posts were established in this general area, which included the Chapleau Post, Biscotasing Post, Flying Post, Missanabie Post, and Peterbell Post. With the exception of the last two, these posts were subsequently abandoned by the Company. "The North West Company also built a post on Missinaibi lake in 1800" (14).

At the close of the century the country was traversed by railroads and the forest began to be opened up by extensive logging and burning. It is reported that many extensive fires were deliberately set by the invading white men. Undoubtedly this change in forest composition has had its effect on the indigenous furbearing mammals. Our present day knowledge of the ecology of many of our native furbearers is so sketchy that it is extremely difficult to evaluate the effects of changes that have taken place in their environment.

Account of Furbearing Species

Order RODENTIA

Castor canadensis—Beaver

The beaver production in Chapleau district has fared well under a regulated harvest (Table I). The steady increase beginning in 1942-43 seems to indicate clearly the value of such a system for producing a sustained annual yield.

TABLE I

THE ANNUAL HARVEST OF BEAVER IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of beaver	1067	798	840	955	1081	1571	1052
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	4.1	4.6	4.7	4.5	4.2	3.5	4.2
Av. value per pelt, $	24.00	26.00	34.00	38.00	56.00	30.00	35.00
Total revenue, $	25,600.00	20,700.00	28,600.00	36,300.00	60,500.00	47,100.00	36,500.00
Av. return value per sq. mi. trapped	5.91	5.67	7.24	8.47	13.22	8.47	8.30

The area necessary to produce one harvested beaver varied only slightly for the period. On the average a little over four square miles of Chapleau country, if properly managed, will produce one harvestable beaver. Perhaps it may be possible to increase the number of animals that may be taken from certain areas, thus reducing the size area required to produce each individual; however, carefully regulated control should be exercised in such an attempt.

Ondatra zibethica—Muskrat

Although the number of muskrats produced in Chapleau district has been relatively small compared to other areas containing more extensive favorable marshes, the harvest of the entire district for the past six years appears to be a fairly direct reflection of fluctuations in the total populations. The size area required to produce one harvested muskrat would seem to be a better reflection of the size of the actual population than the total number taken from the district because of the variation in the amount of land actually trapped (Fig. 3). The fluctuation in numbers has not been violent since each trapper took, on the average, between 25 and 46 animals annually with an average of 37 pelts (Table II); however, a general downward trend is suggested, which could be forecast by the study of past fluctuations in Provincial production (1).

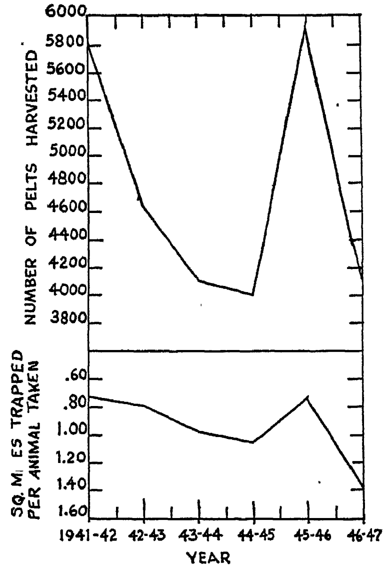

FIG. 3. *Top. Total muskrat produced from Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Order CARNIVORA

Canis latrans—Brush Wolf, Coyote

Brush wolves seem to have entered the Chapleau district rather recently. According to Cross (4) they probably came from the west between 1900 and 1936. They have never become abundant in the area and only recently

TABLE II

THE ANNUAL HARVEST OF MUSKRAT IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of muskrat	5857	4613	4122	4009	5906	4118	4771
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	0.74	0.79	0.98	1.07	0.77	1.35	0.92
Av. value per pelt, $	2.00	2.00	2.25	2.75	3.40	2.00	2.45
Total revenue, $	11,700.00	9200.00	9300.00	11,100.00	20,100.00	8200.00	11,600.00
Av. return value per sq. mi. trapped	2.70	2.52	2.35	2.60	4.38	1.48	2.65

have we been able to obtain valid records of their occurrence substantiated by specimens. On Feb. 21, 1947, one specimen was killed by a train in Delmadge Township and turned over to the Royal Ontario Museum of Zoology. Since then several additional specimens have been obtained from the Chapleau district. There is little value in their pelt on current markets.

Canis lupus—Timber Wolf

Timber wolves are not uncommon in the Chapleau district. They do not constitute a noteworthy fur resource since there is a very poor market for this type of fur in Ontario.

Vulpes fulva—Red, Cross, and Silver Fox

The number of foxes has been increasing across Canada and at the same time showing cyclic fluctuations (3, 5). The harvest from Chapleau is given by color phases in Table III. As shown by Cross (6) and Butler (3), the color ratios vary from one geographical area to another with fairly consistent reduction in the percentage of occurrence of silver and cross phases in recent years. When comparing the color ratios of foxes of the Chapleau district with those of the two nearest Hudson's Bay Company posts listed by Cross (6) (Table IV), closer agreement is found with ratios from Gogama to the east than from Missanabie to the northwest.

Ursus americanus—Black Bear

Bear in the Chapleau district increased until 1945, then apparently decreased. The entire take of bear reported for 1944-45 in all of Ontario amounted to only 333 skins (1). Since the average values of these pelts amounted to only three dollars it is quite understandable why few are skinned for pelts. No record of sale of bear skins was made for the Chapleau area during 1941-47.

TABLE III

THE ANNUAL HARVEST OF FOX IN THE CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of red fox	198	315	333	304	389	375	319
Total catch of cross fox	36	36	21	32	34	24	31
Total catch of silver fox	—	4	1	6	5	1	3
Total catch all types fox	234	355	355	342	438	400	352
Sq. mi. trapped per animal unit harvested	18.5	10.3	11.1	12.5	10.7	13.9	12.5
Av. value per pelt red fox	5.00	6.00	6.25	7.25	8.75	4.00	6.30
Av. value per pelt cross fox	17.00	15.00	20.00	15.00	17.00	13.00	16.00
Av. value per pelt silver fox	18.00	18.00	18.00	18.00	25.00	18.00	20.00
Total revenue (all foxes)	1600.00	2500.00	2500.00	2800.00	4100.00	1800.00	2550.00
Av. return value per sq. mi. trapped (all foxes)	0.37	0.69	0.64	0.65	0.90	0.33	0.58

TABLE IV

COMPARATIVE RATIOS OF COLOR PHASES IN RED FOX (*Vulpes fulva*)

Area	Period	Red fox	Cross fox	Silver fox	Bastard fox
Chapleau district	1941-46	90.5	8.6	0.8	—
Gogama post	1916-20	91.3	8.6	—	—
	1934-38	92.9	5.7	0.5	0.7
	1916-35	86.6	10.1	3.0	0.1
Missanabie post	1916-20	81.1	16.2	2.5	—
	1934-38	84.7	12.3	0.7	2.1
	1916-35	83.4	13.7	2.0	0.7
Ontario	1916-20	71.1	23.9	4.4	0.4
	1934-38	78.0	18.7	2.2	0.9

Procyon lotor—Raccoon

The raccoon is a rare animal as far north as the Chapleau district. None was sold on the market from this area, although one was shot along the Wakami River in Benton Township in November, 1946, by a resident of Chapleau, and examined by us. It was reportedly mistaken for a "young wolf" and the pelt turned in to the Chapleau district office.

Mustela erminea—Ermine, Weasel

The Chapleau production of ermine (Table V) is comparatively small. The area involved in producing one harvested animal unit in this case probably has little significance since the average value of the pelts is so low. The trapping pressure for this species is only sporadic for the main efforts are directed toward more valuable furs.

TABLE V

THE ANNUAL HARVEST OF ERMINE IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of ermine	234	294	500	531	631	593	464
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	18.5	12.4	7.9	8.0	7.2	9.4	9.5
Av. value per pelt, $	0.50	0.55	0.80	0.90	1.30	1.30	1.00
Total revenue, $	117.00	162.00	400.00	478.00	820.00	771.00	458.00
Av. return value per sq. mi. trapped, $	0.03	0.04	0.10	0.11	0.18	0.14	0.10

Mustela vison—Mink

The production of mink has been fairly constant in the Chapleau area in recent years. A low for the period was the first year, 1941-42, when only 555 pelts were taken at the rate of one animal for each 7.8 square miles trapped. This was followed the second year, 1942-43, by a high for the period when 770 pelts were taken at the rate of one animal for each 4.7 square miles trapped. The rate for the remaining four years (Table VI) remained near the six year average of one animal for each 6.8 square miles trapped.

Martes americana—Marten

The trend in production of marten has been most encouraging in the Chapleau district (Table VII). There was almost a steady increase in numbers taken during the six year period 1941-42 to 1946-47 from a low of 58 to a high of 241. The area required to produce each individual (Fig. 4) is only a partial indication of the relationship of trapping pressure to the total numbers taken. The increasing in value per pelt undoubtedly affected a selective trapping pressure upon this species. It is of considerable interest to examine the distribution of the catch of marten as shown in Fig. 2 for the season 1945-46. If a line is drawn across the district dividing equally the trapping areas of the southeastern portion from the northwestern portion, it will be found that 98% of the marten are taken in that half of the district

TABLE VI

THE ANNUAL HARVEST OF MINK IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of mink	555	770	613	591	603	750	647
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	7.8	4.7	6.4	7.2	7.6	7.4	6.8
Av. value per pelt, $	12.00	11.00	18.00	20.00	33.00	22.00	19.00
Total revenue, $	6660.00	8470.00	10,900.00	11,800.00	19,900.00	16,500.00	12,400.00
Av. return value per sq. mi. trapped	1.54	2.32	2.77	2.76	4.35	2.97	2.82

TABLE VII

THE ANNUAL HARVEST OF MARTEN IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of marten	58	84	91	88	185	241	124
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	75	43	43	49	23	23	35
Av. value per pelt, $	33.00	30.00	39.00	42.00	50.00	40.00	41.00
Total revenue, $	1910.00	2520.00	3550.00	3700.00	9250.00	9640.00	5100.00
Av. return value per sq. mi. trapped, $	0.44	0.69	0.90	0.86	2.01	1.73	1.16

nearest the Chapleau Game Preserve. The northwestern portion contains more extensive stands of mature conifers, which presumably creates a more favorable habitat for this species, but in spite of these ecological differences there is strong evidence that some animals are emigrating from the preserve to surrounding territory. If emigration is not a major factor, and these animals are being produced in the area where taken, the actual area required to produce one harvestable marten would be reduced 50% for northern Chapleau district. Seemingly the game preserve possesses value as a reservoir for such furbearers.

On the whole the decrease in marten since 1919 has been alarming. In recent years the provincial output of marten has decreased over 75% from the 1919-20 level (1). If overtrapping has been one of the primary causes of reduction, it may be possible that the registered trapping ground system, which has recently been inaugurated throughout Ontario, will forestall further decrease. If the trend of increase in the Chapleau remains a real one, certainly its trapping system could be cited as a favorable factor.

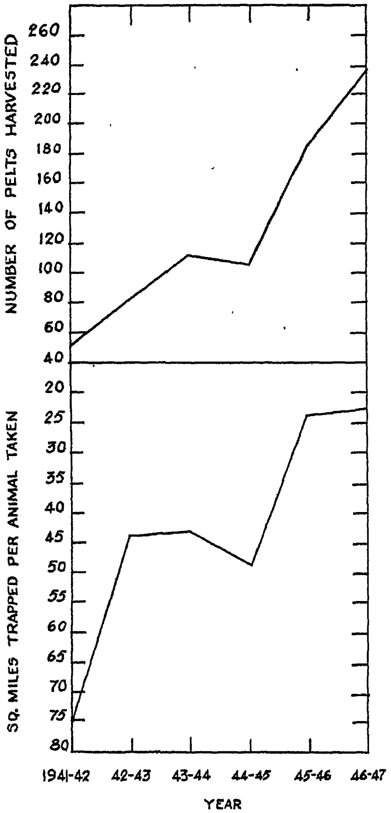

FIG. 4. *Top. Total marten production of Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Martes pennanti—Fisher

Like marten, the fisher showed an encouraging increase (Table VIII) from 1941-42 to 1946-47. Again the area required to produce one trapped animal should give a check on relative increase (Fig. 5). If, however, the district is divided, as suggested for the case of marten, 84% of all fisher caught in 1945-46 would be taken from the northwestern half (Fig. 2). As with the marten this suggests an overflowing effect from the game preserve.

The recent Ontario and Dominion production of fisher has been greatly reduced over past high years (1). Whether or not the trend of increase as shown by the Chapleau district will be reflected throughout the Province remains to be seen. Compared with figures for Ontario totals available for the same years, figures for the Chapleau district show that it is undoubtedly one of the better fisher producing areas of the Province.

TABLE VIII

THE ANNUAL HARVEST OF FISHER IN THE CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of fisher	20	17	22	45	56	92	42
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	216.0	215.0	179.0	95.0	82.0	60.0	104.0
Av. value per pelt, $	53.00	45.00	68.00	70.00	63.00	53.00	59.00
Total revenue, $	1060.00	765.00	1500.00	3150.00	3530.00	4880.00	2480.00
Av. return value per sq. mi. trapped, $	0.24	0.21	0.38	0.74	0.77	0.88	0.56

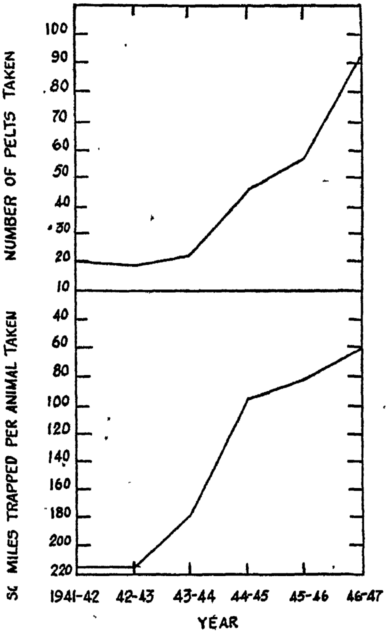

FIG. 5. *Top. Total fisher produced in Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Gulo luscus—Wolverine

According to the older settlers the last record of wolverines in Chapleau district was between 1885 and 1900. Wolverine were probably never very abundant in Ontario. The remaining few animals are restricted to the wilder portions of the far northwestern part of the Province. It would appear that the wolverine is doomed to extinction in Ontario. Since 1928-29 the annual total take has been less than 10 animals in all Ontario (1).

Lutra canadensis—*Otter*

The total number of otter taken from the Chapleau district increased steadily from 1941-42 to 1946-47 (Table IX). However, the size of unit area trapped to produce each pelt indicates some fluctuations in populations

TABLE IX

THE ANNUAL HARVEST OF OTTER IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of otter	63	66	68	70	125	131	87
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	69	55	58	61	37	42	50
Av. value per pelt, $	20.00	17.00	25.00	28.00	30.00	35.00	26.00
Total revenue, $	1260.00	1120.00	1700.00	1960.00	3750.00	4580.00	2400.00
Av. return value per sq. mi trapped, $	0.29	0.31	0.43	0.46	0.82	0.82	0.55

(Fig. 6). The distribution of catch for 1945-46 (Fig. 2) indicates a fairly even population throughout the district. The provincial catch of otter has remained fairly constant since 1919-20 (1), which apparently indicates that the otter is holding up well under existing trapping pressures.

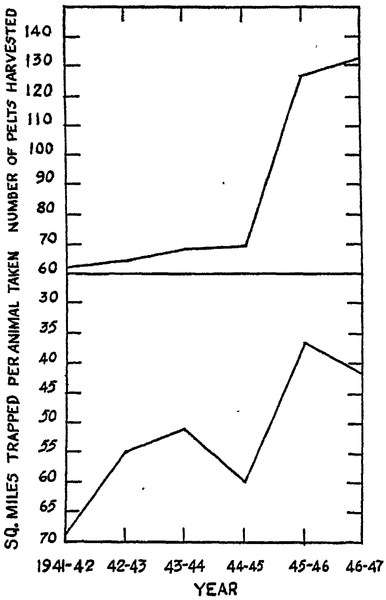

FIG. 6. *Top. Total otter produced in Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Mephitis mephitis—Striped Skunk

The recorded number of pelts taken from the Chapleau district gives no indication of the relative size of populations nor trends of abundance. The low price, coupled with the disagreeable task of handling, brings a very small fraction of available skunk pelts to the market. The average annual catch from 1941-42 to 1946-47 was only 19 skunks.

Lynx canadensis—Canada Lynx

The catch of lynx in the Chapleau district has been a small and variable one in recent years (Table X). MacLulich (11) plotted the lynx returns for Canada from 1751 to 1932. He found violent fluctuations in numbers trapped and demonstrated a cycle with peaks of abundance occurring on the average of every 9.7 years. He also (12) demonstrated a close correlation of the abundance of lynx with that of the varying hare (*Lepus americanus*). The number of lynx taken in the Chapleau district is so small (Fig. 2 and Table X) and the unit area necessary to produce one animal unit so variable that it is doubtful that the graphic indications for only six years can indicate any significant trend in productivity; however, a general increase seems evident, an increase that could be forecast by examination of provincial trends (1). The figures (Fig. 7) do indicate the erratic nature of lynx populations within a small area.

TABLE X

THE ANNUAL HARVEST OF LYNX IN THE CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of lynx	8	6	14	15	8	32	14
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	541	609	282	286	572	174	317
Av. value per pelt, $	37.00	40.00	50.00	47.00	50.00	42.00	44.00
Total revenue, $	296.00	240.00	700.00	705.00	400.00	1340.00	614.00
Av. return value per sq. mi. trapped, $	0.07	0.07	0.18	0.16	0.09	0.24	0.14

Lynx rufus—Bobcat

Although we have no specimens from the Chapleau district, we have obtained reports of four bobcats taken since 1919. Mr. Sam Chappise reported killing one in the district in 1919; Mr. Wilfred McAuley of Chapleau;

one in Lackner Township shortly afterward; Mr. Ignace Mamyguess of Chapleau, one in 9H Township in 1936; and Mr. Alec Bain, one in Rollo Township in 1942. Bobcats are quite rare as far north as Chapleau. However, one pelt was received at the Hudson's Bay Post at Pagwa River in 1946 and presented to the Royal Ontario Museum of Zoology by Mr. W. Glennie, Manager of the post. This animal was a female, taken Nov. 2, 20 miles east of Pagwa River Post, some 40 miles north of the township of Rogers. This area lies 200 miles north of Chapleau. Mr. Glennie reports that to his knowledge no Indian or white trapper had ever heard of a bobcat in that area before.

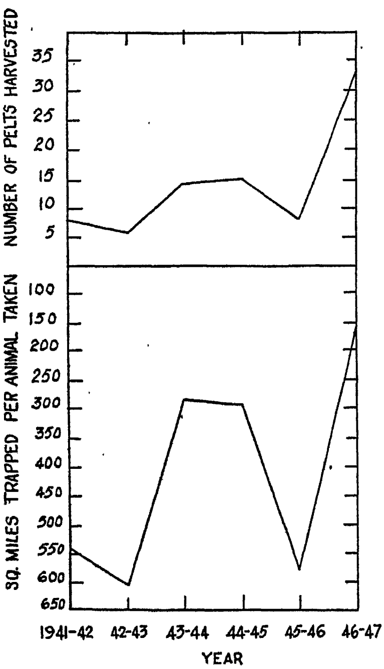

FIG. 7. *Top. Total lynx production of Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Discussion

Two common species, the snowshoe rabbit or varying hare (*Lepus americanus*) and red squirrel (*Tamiasciurus hudsonicus*) do not constitute important fur resources in the area discussed, although an unknown number are taken by trappers each year. Hess (9), however, included the number of these species taken on an 89½ mile trap line over a 13 year period 1931-44 in the Oba-Hearst area, which lies some 100 miles north of Chapleau. Since the Province of Ontario requires no royalty on these species it is difficult to appraise the present or potential values of these two mammals.

A comparative summary of production of the principle furbearers of the Chapleau district is given in Table XI. It will be seen that beaver, muskrat, and mink constitute the more important species of the area in both numbers taken and in total value.

TABLE XI

COMPARATIVE SUMMARY OF PRODUCTION OF THE PRINCIPAL FURBEARERS OF CHAPLEAU DISTRICT

Species	Sq. mi. trapped per animal harvested			Catch per trapper (one twp.)			Return value, $, per sq. mi. trapped			Av. total revenue,	Av. total catch
	Av. low	Av. high	Six yr. av.	Av. low	Av. high	Six yr. av.	Av. low	Av. high	Six yr. av.	Six yr. av.	Six yr. av.
Beaver	3.5	4.7	4.2	7.2	9.6	8.2	5.67	13.22	8.30	36,500.00	1052
Muskrat	0.7	1.3	0.9	25.3	46.8	37.2	1.48	4.38	2.65	11,600.00	4771
Red fox	11.6	21.9	13.7	1.6	2.9	2.5	0.23	0.74	0.46	2000.00	319
Cross fox	101.0	232.0	144.0	0.15	0.33	0.24	0.06	0.15	0.11	490.00	31
Silver fox	—	5563.0	1550.0	—	0.05	0.02	—	0.03	0.01	57.00	3
Ermine	7.2	18.5	9.5	1.9	4.7	3.6	0.03	0.18	0.10	458.00	464
Mink	4.7	7.8	6.8	4.4	7.1	5.0	1.54	4.35	2.82	12,400.00	647
Marten	23.0	75.0	35.0	0.5	1.5	1.0	0.44	2.01	1.16	5100.00	124
Fisher	60.0	216.0	104.0	0.16	0.56	0.3	0.21	0.88	0.56	2480.00	42
Otter	37.0	69.0	50.0	0.5	0.9	0.7	0.29	0.82	0.55	2400.00	87
Lynx	174.0	609.0	317.0	0.05	0.16	0.1	0.07	0.24	0.14	614.00	14

Consideration of areas of comparative size required to produce each harvested animal unit should be useful in connection with future conservation and management of our furbearers and might well be the basis for further research.

Since trapping returns form a substantial part of the total income of many residents in northern Ontario, it is important to maintain the fur resources in continued production. The decrease in the number of certain of the fur-bearing mammals threatens one of Canada's most important heritages. Now is the time to follow up the important conservation policies already adopted by further measures based on both intensive and extensive research so that the fur production of Ontario and Canada may be maintained on the highest possible level.

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THE EFFECT OF DIETARY FAT ON THE HEAT TOLERANCE OF GOLDFISH (*CARASSIUS AURATUS*)[1]

BY W. S. HOAR[2] AND J. E. C. DORCHESTER[3]

Abstract

Three groups of goldfish were fed diets containing 25% fat. The diets for the three groups differed in the degree of unsaturation of these fats. Pilchard oil (iodine value 181.7), herring oil (iodine value 128.4), and lard (iodine value 66.2) were the fats used. Heat tolerance of the fish was then tested by holding them at a constant high temperature and observing the time of death. Variations in the ability of the groups to withstand high temperature were correlated with differences in the degree of unsaturation of their extracted fats. The iodine value of the herring oil diet was very close to that of the fats of the goldfish prior to feeding and no change in the value for fish fed on this diet was observed. Each of the other two diets changed the degree of unsaturation of the goldfish fats by approximately 54% of the maximum theoretical change. These changes modified the heat resistance of the fish. The degree of modification of heat tolerance, however, cannot be strictly correlated with the degree of change of unsaturation of fats and it is concluded that factors associated with differences in melting point rather than melting point of the fat itself are probably involved in heat resistance.

Introduction

Several writers have suggested that the action of heat on the lipids of the cell may be an essential feature of heat death (1, 6). This lipoid liberation theory of heat death is based on the observations that animals living at lower temperatures or raised at lower temperatures have fats with lower melting points than those living at higher temperatures or raised at higher temperatures. Pertinent references are cited in the reviews mentioned above. Heilbrunn (6) uses these observations in connection with his calcium release theory to explain heat death. According to this explanation the melting of the fats releases them from their combination with the proteins and calcium that are essential constituents of every cell membrane. Calcium released from the cell cortex to the interior of the cell brings about coagulation and death. The presence of fats of higher melting points in the cortex is responsible for increased resistance to heat. Acclimatization to heat is dependent upon the development of fats with higher melting points. Whether this explanation is accepted in detail or not, there is an apparent relation between the nature of fats and temperature at which the animal lives.

In this work an attempt has been made to test this theory by altering the melting point of the fats of goldfish and determining the effect of this treatment on the heat tolerance of the animals.

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Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C., with financial assistance from the National Research Council of Canada and British Columbia Packers Limited.

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Materials and Methods

These experiments are based on 265 goldfish (*Carassius auratus*) obtained from the Goldfish Supply Co., Stouffville, Ont. The fish ranged from 10 to 15 gm. in weight. They were divided into groups and fed diets containing 25% fat. Three different fats* were used: pilchard oil (iodine value 181.7), herring oil (iodine value 128.4), and lard (iodine value 66.2). As a base for these diets, 70% pablum (Mead Johnson Company of Canada, Ltd.) was used and the fat was bound to the pablum with lecithin (5%). The fat and lecithin were first dissolved separately in peroxide-free ether and then mixed. The solution of fat and lecithin was then poured into the pablum, mixed thoroughly, and the ether extracted *in vacuo* at 37° C. Fresh diets were prepared each week and stored in a refrigerator. The iodine values of the diets thus prepared were determined as follows: pilchard oil diet, 159; herring oil diet, 115; lard diet, 64. The stability of these diets was tested by determining the iodine values of the fats extracted from diets one week old and two weeks old. The change in value did not exceed one unit during this period.

The fish were fed, as a group, an amount of food equivalent to 0.3 gm. per fish per day. This amount was divided into three feedings and each feeding lasted approximately 30 min. Only a portion of the food was placed in the aquarium at one time so that the animals could eat as much as possible before it sank to the bottom and disintegrated. It was necessary to change the water and clean the aquaria once per week.

The fish were kept in glass aquaria. The water was aerated and the temperature maintained at 20° ± 0.5° C. To determine heat resistance, the fish were held at a constant high temperature. For this a large tank divided into three compartments with plastic netting was available. Thus, the three groups could be subjected to identical conditions for this test.

The iodine values of the oils were determined by the Wij's method. The extraction procedure consisted of macerating the fish in a Waring Blendor, drying it *in vacuo* at 45° to 55° C., and extracting it in an A.S.T.M. extraction apparatus with peroxide-free ethyl ether for two hours at 38° C. Following the extraction the ether was distilled off *in vacuo* at 38° C. and the oil dried in a vacuum desiccator.

Results

Effect of Dietary Fat on the Body Fats

The data are summarized in Table I and presented graphically in Fig. 1. The herring oil diet produced little change in the degree of unsaturation of the goldfish body fats.

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* *The fish oils were supplied by Western Chemicals, Ltd., through the courtesy of Dr. W. Chalmers. The lard was procured from Burns and Co. Ltd. through Dr. B. E. Bailey. These were stored in the frozen condition at the Pacific Fisheries Experimental Station of the Fisheries Research Board of Canada. We are indebted to Dr. Bailey for numerous helpful suggestions.*

TABLE I

AVERAGE IODINE VALUES FOR GOLDFISH FED DIETS CONTAINING FATS OF DIFFERENT DEGREES OF UNSATURATION

Duration of feeding, days	Fish in each diet group	Iodine values		
		Pilchard oil diet (I.V. 159)	Herring oil diet (I.V. 115)	Lard diet (I.V. 64)
0	11*	—	113.5	—
6	6	122.3	115.5	93.4
12	6	124	112.9	90.4
21	6	126	115.6	—
29	8	128.2	112.6	88.4
32	4	133.4	112.9	86.5
34	6	136	111.4	89.2
37	6	136.1	113.1	86.4
46	6	137.1	113.2	91.38
55	8	137	110	85.8

* *Entire sample.*

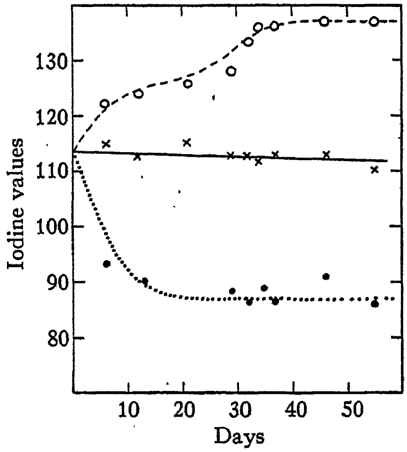

FIG. 1. *Changes in degree of unsaturation of body fats of goldfish fed pilchard oil (broken line), herring oil (solid line), and lard (dotted line).*

This was expected from a comparison of the value for prefed fish (113.5) with the iodine value of this diet (115). The pilchard oil diet and the lard diet produced very definite changes in the degree of unsaturation of the goldfish fats. The change was more rapid and reached its maximum value more quickly in the case of the solid fat (12 days as compared to 34 days). In both cases the value became constant at about 54% of the maximum theoretical change possible if the fats of the fish were entirely altered to those of the diet.

Effect of Dietary Fat on Heat Tolerance

The histogram of Fig. 2 shows the temperature tolerance of the different groups as measured by the time required to kill 50% of a sample of 90 goldfish (30 from each diet group) at a temperature of 36° C. Three of the fish on the lard diet lived 24 hr. (and longer) at 36° C. The maximum survival time for fish on the herring oil diet was 80 min.; for those on the pilchard oil diet 137 min. These fish had been fed on the high-fat diets for 46 days and consequently the change in body fats had reached a maximum. The contrast in heat tolerance between the solid fat (lard) and the liquid fats (herring or pilchard oil) is evident. Moreover, the order of heat resistance is that which would be expected if heat tolerance is related to melting point of fats. However, the correlation between iodine value (hence melting point) of the body fats and heat tolerance is not precise. The iodine values form an evenly graduated series (85, 110, 137) but there is a very small difference between the lethal times of the fish fed herring oil and pilchard oil (35 min. compared to 33.5 min.).

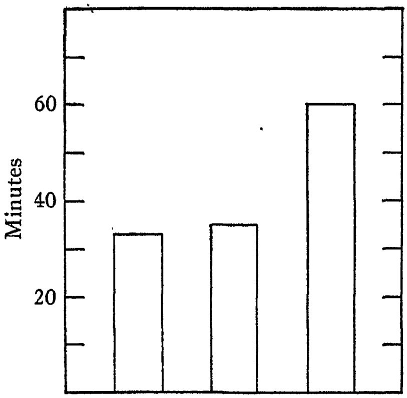

FIG. 2. *Time required to kill 50% of a sample of goldfish fed on three diets differing in fat content. Diets from left to right are pilchard oil, herring oil, and lard.*

In Fig. 3 the data for this experiment have been treated in another way. The lines show the rates at which the fish died during the first 75 min. at 36° C. The order again is the order anticipated if there is a relation between melting point of fats and heat tolerance. However, the fiducial limits (99% level of confidence) of the regression coefficients show that there is no statistical difference between the behavior of the samples on the two fish-oil diets but that the group on the solid diet (lard) is distinct.

A preliminary series of temperature tolerance tests involving approximately 100 fish demonstrated essentially the same features as shown above. These results are not presented in detail since the fish fed the pilchard oil diet had not been fed long enough to produce the maximum change in their body fats. The lethal times (50% level) and rates of dying for the goldfish fed lard and herring oil were almost identical while the fish fed pilchard oil survived much longer than those of the experiments above. This difference is readily explained by the fact that the pilchard oil diet had produced only a partial change in the goldfish fats at the time of the tests.

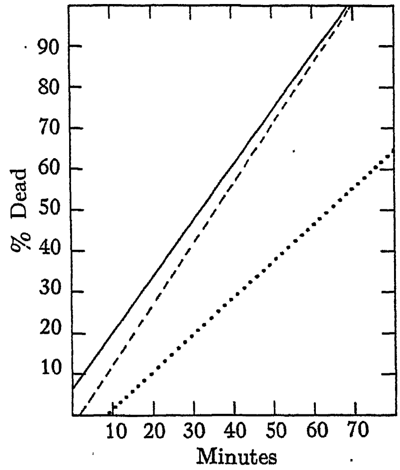

FIG. 3. *Regression lines to show the rate at which goldfish fed three different diets were killed at 36° C. Solid line—herring oil diet (b = 6.82 ± 1.08); broken line—pilchard oil diet (b = 7.34 ± 1.32); dotted line—lard diet (b = 4.42 ± 0.293).*

Discussion

It is evident that the degree of unsaturation of the body fats of the goldfish can be modified by dietary fat. This fact has been frequently demonstrated for mammals (9, 10) and was found by Lovern (10) to be true for the eel (*Anguilla vulgaris*) if the diet was high in fat (20.7%). The present experiments show that the fats of goldfish are altered more readily with lard than with pilchard oil although the degree of unsaturation of these two fats differ about equally from the goldfish fat prior to feeding. Deposition of dietary fat in mammals likewise varies with the nature of the fat (9). In the present experiments, the maximum change produced in the goldfish is only about 54% of the theoretical.

Further, it is apparent that the heat tolerance of goldfish can be modified by dietary fat. The greatest effect, obtained with the lard diet, corresponds to an acclimation temperature of about 24° C. or a change in acclimation temperature equivalent to 4° C. (5).

The data demonstrate a general relationship between degree of unsaturation of the body fats and heat tolerance. If, however, heat tolerance is dependent upon the melting point of the body fats there should be a strict correlation between these two factors. This is not apparent. The pilchard oil and herring oil groups behave very similarly. If melting point is the important factor, it must be concluded that the difference between the melting points of the fats of fish fed herring and pilchard oil is not great enough to change the heat tolerance. It seems more likely that some other property of these fats is involved. These analyses are based on total ether extract. The nature of the phospholipid fats or other ether soluble materials may be more important. Variations in the phospholipid fat have been demonstrated for poikilothermic animals in relation to temperature of the environment (12, 7). Another possibility is that the different fats bring about changes in the water content of the tissues (11) or permeability of the cell membranes to water (2) and thus affect the heat tolerance.

The failure to show a precise correlation between the melting point of the body fats and heat resistance is in line with experiments on temperature acclimation of goldfish (8) and the breeding of blowflies at different temperatures (4).

In connection with the present experiments it would be interesting to know the lethal temperature relations of pilchard (*Sardinops caerulea*) and herring (*Clupea pallasii*) since the former is a more southerly fish although its fat has a lower melting point. This is contrary to the expectation from findings for many other species where the animals living in the lower temperature environment have the fats with lower melting point (references previously cited).

As yet the temperature relations of these groups of experimental fish have not been completely worked out. Observations on the behavior of the fish when subjected to high temperature indicate that the herring and pilchard oil diets had definite effects on the fish that cannot be demonstrated by lethal times and rates. Further experiments are in progress. It may be mentioned, however, that the initial reactions of fish fed on herring oil are more violent than those fed on pilchard oil. In several cases fish fed herring oil died immediately on being placed in the warm water. This phenomenon was not observed with the other fish. The herring oil diet appears to produce nervous symptoms in the fish even at room temperature. Brown (3) observed nervous symptoms in pigs fed on diets containing 14% menhaden oil although the fish oil acids had not accumulated in the brain. From these observations it is felt that the herring and pilchard oil diets have produced changes in temperature relationships not demonstrated by the lethal times.

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THE BIOLOGY OF *CEPHALONOMIA WATERSTONI* GAHAN[1]

BY R. OMAR RILETT[2]

Abstract

Life history and morphological studies were made on *Cephalonomia waterstoni* Gahan, a hymenopterous parasite on the larval and pupal stages of the rusty grain beetle, *Laemophloeus ferrugineus* (Steph.). At 90° F. and 65 to 75% relative humidity the whole life cycle of *C. waterstoni* is completed within 12 to 13 days. The adult life span of females is about three weeks, while that of males is much shorter. The male, which prior to this work was unknown, can easily be distinguished from the female by its smaller size, its less rectangular-shaped head, and its longer antennae. The first flagellar segments of the males are brownish-black in color while those of the females are yellowish. The ratio of females to males is somewhere near 2 : 1. The ease with which the parasite *C. waterstoni* can be reared in large numbers in the laboratory, and its remarkable ability to check infestations of *L. ferrugineus* under experimental conditions, indicate that this species might be of value in biological control programs for *L. ferrugineus* in warehouses, mills, and elevators.

Introduction

Cephalonomia waterstoni Gahan, a minute parasitic wasp belonging to the family Bethylidae, was first described by Gahan in 1931 (1). The description of the species was made from 11 female specimens taken at quarantine in Washington, D.C. by E. A. Back from a shipment of grain the original source of which was said to be Australia; from four females collected from stored corn at Baton Rouge, La., by C. O. Hopkins; and from one female taken at Urbana, Ill., by W. V. Balduf.

Gahan (1) examined numerous female specimens of this species now in the British Museum and these are the same insects as those listed in the Royal Society's 9th Report of the Grain Pests (War) Committee, 1921, listed under the name *Cephalonomia* sp. (p. 50) and comprising the lots numbered 247, 262, 266, 347, 348, 349, 370, 384, 386, 389, and 390 of that report.

In 1931 the species had not yet been associated with any definite host, but it was thought that it was probably parasitic upon one or more of the Coleoptera that infest stored grain.

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Since 1931 it has been established that *Cephalonomia waterstoni* is a parasite on the larvae of *Laemophloeus minutus* Ol. (*pusillus* Schonh.) (2) and on the larvae of *Laemophloeus ferrugineus* (Steph.) (4).

This parasite has been taken in considerable numbers by the writer in grain from a flour mill at London, Ont., and in grain from the University of Wisconsin feed storage building. The wasp appeared in the cultures of *Laemophloeus ferrugineus* being kept in the Zoological Laboratory at Madison during the summer of 1944, shortly after the original culture had arrived from Canada. Thus it is not known whether the parasite became established in the beetle cultures after reaching Madison or before leaving Canada.

Mr. C. F. W. Muesebeck, Washington, D.C., who identified specimens from cultures used in this investigation, informed the writer by letter that practically nothing was known concerning the biology of *Cephalonomia waterstoni*. It was therefore decided to supplement the study of *Laemophloeus ferrugineus*, which was then under way, with a study of the biology of its parasite, *Cephalonomia waterstoni*, paying special attention to the possibilities of using the latter as an agent in the biological control of the former.

Life History of *Cephalonomia waterstoni* Gahan at 90° F. and 65 to 75% Relative Humidity

Cephalonomia waterstoni females lay their eggs on the larvae and in some instances the pupae of *Laemophloeus ferrugineus* (Steph.). The fourth instar larva is the stage usually parasitized, no eggs having been found on the first or second instars, although the female parasite attacks the younger larvae just as ferociously as she does the older ones. Usually one or two (rarely three) eggs are deposited on a single host larva. Of 408 parasitized larvae examined, 210 had one egg, 190 had two eggs, and eight had three eggs adhering to them.

The eggs are almost always deposited just caudad to the host's metathoracic legs on the ventral lateral margin of the first two or three abdominal segments (Fig. 1). However, eggs may be found anywhere on the ventral, lateral, or dorsal sides of the abdomen or thorax of the host.

The gravid female wasp searches for a suitable larva upon which to oviposit and, when she finds one, attacks it immediately. Once her powerful mandibles have closed upon her prey its escape is impossible. Although the larva is much larger than its attacker, all of its twisting and lashing movements, which throw the attacking female violently to and fro, are to no avail, and it finally is stung into submission. The struggle may last for several minutes, during which the parasite may make several attempts to pierce the larva's integument with her slim, lancelike ovipositor, which functions primarily as a poison-injecting instrument (Figs. 26 and 27), before she is successful in stinging her victim. The stung larvae, apparently paralyzed, is carried off by its conqueror to a secluded spot where oviposition takes place. In the rearing cages, where there was no place in which to hide, the female wasp would restlessly carry the paralyzed larva around the cage, and often she would continue this fruitless activity after she had deposited eggs upon it. Infrequently the paralyzing effect of the sting was not permanent and the parasitized larva, with the eggs adhering to it, would become active and crawl about in the cage.

The stinging apparatus does not function as an ovipositor, although it has a function at the time of oviposition. When the egg is being laid the first and second valvulae of the stinging apparatus are extended posteriorly and the egg emerges from the female genital duct ventral to the valvulae. The valvulae are used to press the egg tightly against the larva, and after the egg is laid they are run back and forth over it to make sure that it is closely appressed to the body of the host. In a short time the egg becomes rather firmly stuck to the exoskeleton of the host larva. It takes a little over a minute for the egg to be laid, and it is forced to the outside by a "shunting" motion of the posterior abdominal segments. The female maintains her oviposition posture upon the larval host for a few minutes after the egg is laid.

The white and almost transparent eggs "hatch" about 30 hr. after they are laid and the parasite larvae reach their full size about 23 hr. later. Thus it is only a little over two days from the time the eggs are laid until the resulting larvae are full grown. Approximately two hundred different individuals were observed daily as they developed from egg to adult. Records of these daily examinations were kept, but the variations in the length of the developmental periods for the different individuals were so small that the detailed data are omitted here. Almost without exception the insect completed its development from egg to adult in 11 or 12 days.

The parasite larva (Figs. 2 and 23) keeps the same position on the host as that occupied by the egg, and since the egg gradually increases in size up to the time of hatching it is very difficult to determine the exact time that the chorion is shed. This difficulty is increased because of the extreme thinness of the chorion, which remains undetectable under a binocular dissecting microscope. The time given above for the hatching of the larva was recorded at the stage of development at which a slight knoblike protuberance appeared at one end of the "egg". This was interpreted as the appearance of the anterior end of the larva from the egg, but whether the chorion actually surrounded the rest of the larva at this time was not determined.

The parasite larva grows very rapidly and reaches its full growth in one day (Fig. 23). It feeds by inserting its anterior end into the host and sucking up the fluid contents of the haemocoel. The host larva must remain alive until the parasite has reached its full larval size or the latter will fail to develop. *Cephalonomia waterstoni* larvae develop so rapidly that when two eggs are laid on the same host, the one that hatches last will usually be smaller than the other (Fig. 23). A full-grown *Laemophloeus ferrugineus* larva provides just about enough food for the complete development of two parasite larvae. In those instances in which three eggs were deposited on a single host larva the first parasite larva to start feeding was usually the only one to complete its development. However, in one case three adult parasites developed upon one host larva. In this instance the parasite larvae all started feeding at approximately the same time and as a result all received the same proportion of food. None of these larvae reached full size and the resulting adults were much smaller than normal.

The number of larval stadia was not determined. Fritz Van Emden (5), working with *Cephalonomia quadridentata* Duchaussoy, reported five stadia for the above species. He determined the different larval instars by measuring the spiracular diameters of the shed exuviae, which accumulated on the ventral side of the fifth instar between it and the host. He found four sets of spiracles of different sizes and concluded that each set represented a different instar. No such study was made on *Cephalonomia waterstoni* but probably the number of stadia would be the same as for *Cephalonomia quadridentata.*

When the *Cephalonomia waterstoni* larvae have reached their full size they leave the host, which has by then been reduced to an empty shell with nothing remaining but the head and collapsed body wall, and spin oval-shaped, white, silken cocoons (Fig. 4). Soon after the completion of the cocoon the larva changes into the prepupal stage (Fig. 5) and a little later it gives off a mass of black fecal material that forms a dark globule at one end on the cocoon. Apparently the contents of the intestine are voided just before pupation. The period from the start of the spinning of the cocoon until the prepupal stage is reached is about two days. The prepupal stage lasts another two days, when ecdysis occurs and the pupal stage appears (Figs. 5 and 6). The pupal stage in turn lasts for five days, when the last molt occurs and the imago emerges. Shortly afterward it chews a circular opening through the cocoon and escapes to the outside.

If males are present the females are mated shortly after they emerge. There is no preliminary courtship behavior. The male, upon encountering a female that is physiologically ready to mate, mounts her abdomen to which he holds with all three pairs of legs. Then bending his abdomen downward and forward he inserts the aedeagus into the female genital opening. Fig. 24 shows the male genital clasping organs, which aid in the copulatory act. Copulation lasts for about a minute to one and one-half minutes. Mated females may give rise to both female and male progeny but unmated females have only male offspring. Adult females when only one day old may start laying eggs. Therefore at 90° F. and 65 to 75% relative humidity the whole life cycle of *Cephalonomia waterstoni* is completed within 12 to 13 days. The adult life span of females is about three weeks, while that of males is much shorter. Virgin females may live long enough to mate with their own offspring. This fact was brought out when a young male was observed in copulation with his previously unmated mother. Therefore it would be possible for a single unmated female to set up parasitic infestation among host larvae by mating with one of her first male offspring, after which time she would be able to produce female as well as male progeny.

Adult *Cephalonomia waterstoni* females apparently feed upon the larvae of *Laemophloeus ferrugineus* for they have frequently been observed chewing captured host larvae. It seems probable that this wasp chews a hole through the cuticula of its prey and drinks the fluid that oozes out through the opening.

Description of Stages in the Life Cycle of *Cephalonomia waterstoni* Gahan

The Egg (Fig. 1)

The white shiny eggs vary from 0.192 mm. to 0.356 mm. in length and from 0.069 mm. to 0.163 mm. in width. This wide variation in the size of different eggs is partly due to the increase in size of the egg after it is laid. The eggs are slightly curved with both ends bluntly rounded. The chorion and vitelline membrane are very thin and structureless, and so transparent that pulsations of the embryo are plainly visible within the egg. The embryo likewise is colorless and transparent, and the pulsations just mentioned appear as rhythmic waves of a slightly opaque fluid moving at regular intervals from one end of the egg to the other.

The Larva (Figs. 2, 3, and 23)

The yellowish-white, somewhat pear-shaped larvae are completely legless, and are not distinctly divided into head, thoracic, and abdominal regions. The head end is narrower than the abdomen, which is bluntly rounded to a point at the anal end. Behind the head three thoracic segments and nine abdominal segments are visible. The segmentation is very indistinct, as shown in Fig. 2. The mouth parts of the head are not sufficiently sclerotized to make them visible even when the larva is stained and mounted on a slide. It is assumed therefore that the mouth parts are very rudimentary, and that the structures that are present are mostly membranous. A protrusion, which probably corresponds to the labium, is visible on the ventral side of the anterior end of the larva.

The abdomen when viewed with reflected light has several white, approximately circular clusters that apparently lie in or just under the cuticula of the abdomen. They appear dark in color when viewed by transmitted light.

The full-grown larvae are from 1.5 to 2.0 mm. in length, although the attainment of normal growth is dependent upon the amount of food available.

In the prepupal stage the shape of the pupa begins to take form; the division of the larva into three body regions, head, thorax, and abdomen, becoming more distinct (Fig. 5).

The Pupa (Figs. 5 and 6)

The pupa, which at first is creamy white in color with a slight greenish tinge to the abdomen, shows the characteristic structures of the adult. The head is bent ventrad and caudad under the prothorax, and the antennae are directed posteriorly and medially, so that their tips almost meet on the ventral side of the anterior segments of the abdomen. The legs lie along the ventral lateral edges of the thorax and extend caudad ventrally along the sides of the abdomen. The anlagen of the two pairs of wings extend ventrally over the second pair of legs in a diagonal direction towards the median and posterior part of the body.

The large compound eyes and ocelli begin to show a light brown pigmentation by the end of the first day of pupation, and by the second day they are quite dark in color. On the third day the abdomen begins to turn black and a "wave" of pigmentation spreads cephalad. By the fourth day the entire pupa is jet black, with the exception of the legs and antennae (Fig. 6). On the fifth day the shiny black adult emerges.

The Adult

The female was described by Gahan as a new species in 1931. Prior to the present work the male was unknown.

The description of the species as given by Gahan follows:

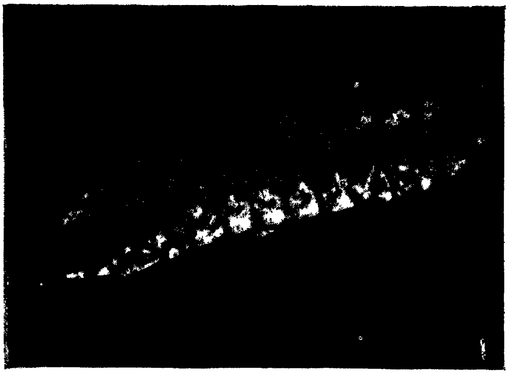
"Female.—Length 1.6 mm. Black and shining; mandibles, pedicel, and first flagellar joint more or less, and all tarsi yellowish; antennal flagellum and the tibiae brownish black; wings hyaline or with a very faint discal cloud. Head viewed from in front longer than broad (20 : 16), the sides nearly parallel or very slightly convex; eyes situated much nearer to mouth than to vertex, about their own length below vertex; ocelli distinct, in an equilateral triangle; whole head finely reticulate-punctate. Antennae inserted at clypeus, 12-jointed; scape two and one-half to three times as long as broad, pedicel about one and one-half times as long as broad, joints 3 to 11 subquadrate, joint 12 about twice as long as broad. Thorax flattened dorsally and with reticulate-punctate sculpture like the head; pronotum about three times the length of mesoscutum, much narrower anteriorly than posteriorly; mesoscutum without longitudinal grooves; scutellum distinctly longer than mesoscutum, with a short groove or pit on either side of base; propodeum flat, in the same plane as scutellum, as long as pronotum, distinctly narrower at apex than at base, sculptured like the thorax, finely margined laterally and at apex of dorsum, a very delicate median longitudinal carina present or absent, the lateral and posterior faces sculptured like the dorsum. Wings well-developed, the prostigma and pterostigma very small, other veins except the submarginal effaced, the forewing without basal cells. Legs normal, the femora moderately swollen, the hind tarsi longer than their tibia. Abdomen ovate, a little broader than the thorax and subequal to it in length, smooth and polished, the third tergite with a barely perceptible suggestion of a depression on each side of the middle.

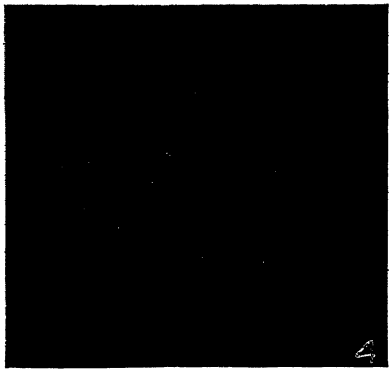
Male.—Unknown.

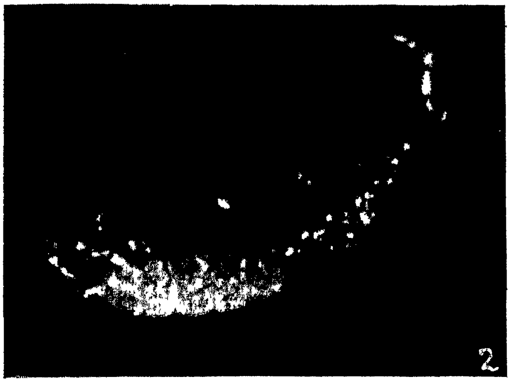
Type locality.—Australia.

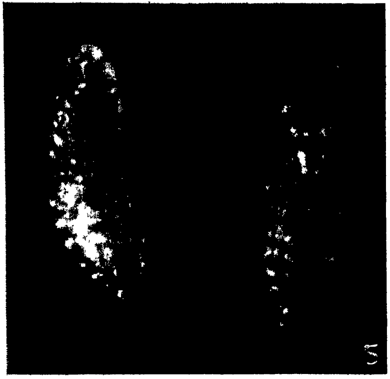
Type.—Cat. No. 43361, U.S.N.M."

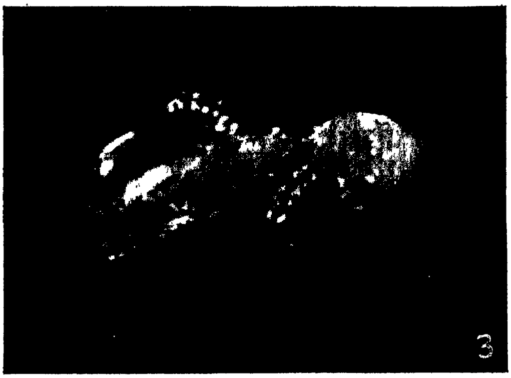
PLATE I











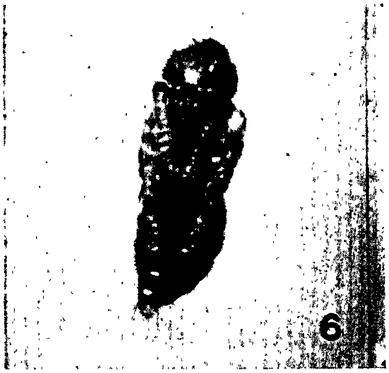


FIG. 1. *Eggs of Cephalonomia waterstoni on a larva of Laemophloeus ferrugineus.*

FIG. 2. *Young larvae of Cephalonomia waterstoni feeding on a larva of Laemophloeus ferrugineus.*

FIG. 3. *Young larvae of Cephalonomia waterstoni feeding on a pupa of Laemophloeus ferrugineus.*

FIG. 4. *Cocoon of Cephalonomia waterstoni.*

FIG. 5. *Prepupa and early pupa stage of Cephalonomia waterstoni.*

FIG. 6. *Late pupa stage of Cephalonomia waterstoni.*

The male can be easily distinguished from the female by its smaller size, its less rectangular-shaped head and its longer antennae. Males have the first flagellar joints brownish-black in color, instead of yellowish as in the case of the female. The male is about three-fourths as long as the female. Table I lists the lengths of 12 males and 12 females. It should be recalled that many adults may be much smaller than normal because of retarded larval development due to a paucity of food. None of these small individuals were included in the measurements listed. The adult is greatly flattened dorsoventrally and the wings when at rest are held so that one pair fits over the other pair along the back of the abdomen. The flattened body and the position of the wings enable both sexes to travel very rapidly through bins of grain.

TABLE I

LENGTH MEASUREMENTS OF 24 *Cephalonomia waterstoni* ADULTS

Males		Females	
Insect number	Length in mm.	Insect number	Length in mm.
1	1.6	1	1.9
2	1.5	2	2.0
3	1.5	3	1.9
4	1.6	4	2.1
5	1.6	5	1.9
6	1.1	6	1.9
7	1.2	7	2.0
8	1.5	8	2.0
9	1.2	9	1.7
10	1.2	10	2.0
11	1.5	11	1.9
12	1.6	12	2.0
Average	1.4	Average	1.9

The head of the female is almost rectangular in shape when viewed from the front (Fig. 9), measuring about 0.43 mm. in length by 0.34 mm. in width, just posterior to the compound eyes. The head of the male is much smaller and less rectangular in shape (Fig. 10) than that of the female, measuring about 0.32 mm. in length by 0.30 mm. in width. The antennae of the male are longer than the more robust antennae of the female (Figs. 7 and 8).

The mouth parts of the male and female are very similar, except that the female structures are somewhat larger (Figs. 11, 12, 13, and 14). The mandibles (Figs. 11 and 14) have well developed bristles on the outer edges and finer bristles on the inner edges. There are three toothlike projections on each mandible. The median pair of teeth are blunt and rounded and fit into the concavity formed by the larger, sharply pointed, lateral tooth, which curves inward over them. The female mandible is about 0.13 mm. long and 0.06 mm. wide, while that of the male is approximately 0.12 mm. by 0.06 mm.

The maxillae and labium are united into a maxillolabial complex (Figs. 12 and 13). The cardo is shaped very much like the stock of a rifle, and most of it is situated inside the head capsule. The stipes is semicircular in shape and articulates with the cardo by a spherical joint. It bears a number of sensory bristles on the lateral and ventral sides. On the outer distal edge is the four-segmented maxillary palp, while the inner dorsal edge bears the triangular lacinia and the almost circular galea, which is narrow at the base and wide at the tip. Both the galea and lacinia are almost completely membranous. The lacinia has a few very fine hairs at its tip, while the galea has numerous strong bristles, some of which are borne on the tip of conelike projections. A pair of papillaelike projections, which may possibly be taste papillae, are also present on the border of the galea. The labium is connected to the head capsule by a Y-shaped submentum. The weakly sclerotized mentum, which beyond the labial palps gradually changes without any boundary into the membranous united glossae, is rectangular in shape. A two-segmented palp arises from each lateral anterior edge, and just anterior and medial to them are the paraglossae. The mentum bears several long bristles as shown in Figs. 12 and 13.

The labial palpi are two-jointed, the distal segment bearing a few long bristles. The glossae have four bristle-bearing sensory cones on the ventral side near the anterior border. In addition the anterior border and the dorsal surface are densely covered with extremely small hairs. The paraglossae lie dorsally on top of the united glossae and are more strongly sclerotized at their distal ends, which bear a row of six or seven strong, bristle-bearing cones.

The well developed, beautifully iridescent wings show practically no sign of venation in either the male or female. The prostigma and pterostigma are very small and the forewing has no basal cells. The costal margin of the forewing bears a few stiff bristles, while the anterior and posterior apical curvatures bear a marginal fringe of hairs. The hind wing has a similar marginal fringe of hairs, which extends along the anal margin of the wing as well. Both fore and hind wings are covered by fine hair over the entire surface.

Head and mouth parts (male and female) of Cephalonomia waterstoni Gahan.

CD: cardo; GA: galea; GL: glossae (united); LBP: labial palp; LC: lacinia; MT: mentum; MXP: maxillary palp; PGL: paraglossa; SMT: submentum; ST: stipes.

FIG. 7. *Antenna (male).*

FIG. 8. *Antenna (female).*

FIG. 9. *Head (female).*

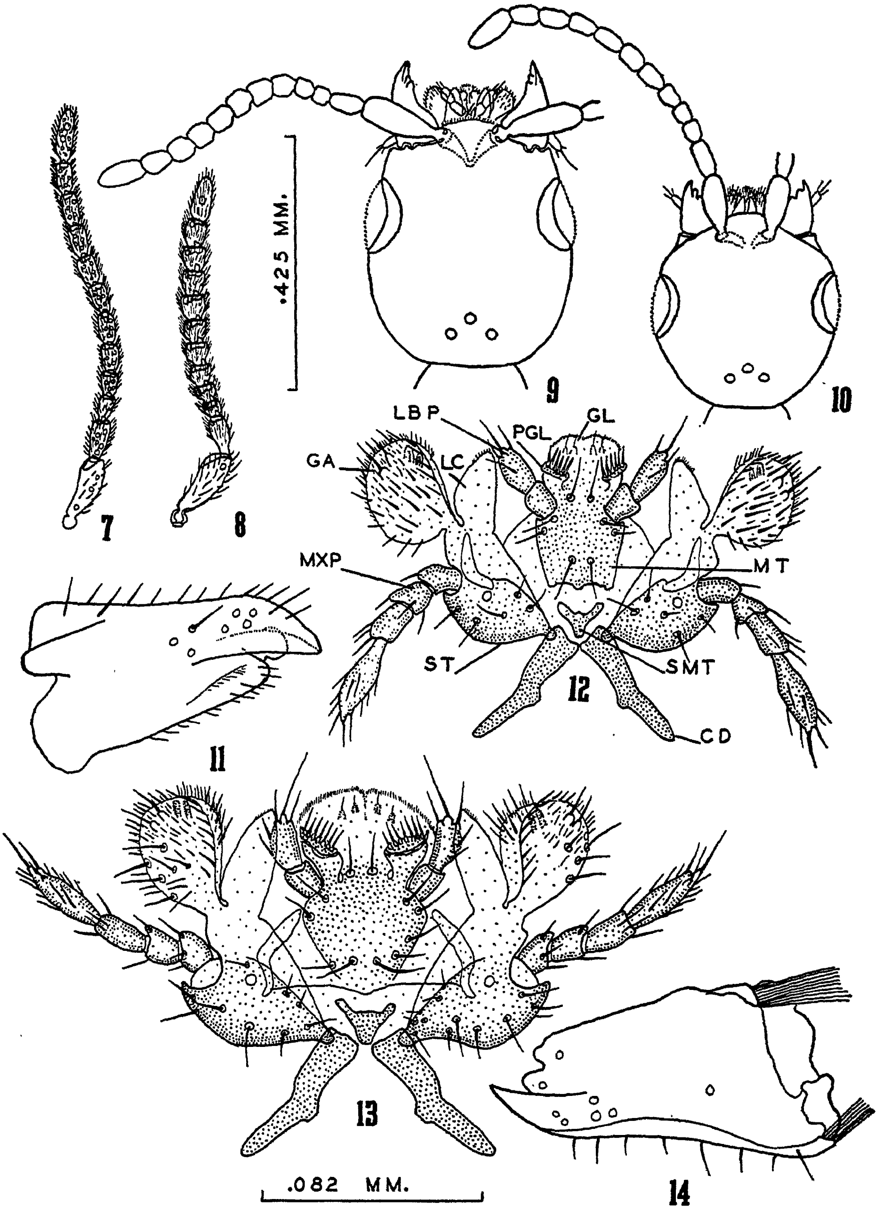
FIG. 10. *Head (male).*

FIG. 11. *Mandible (male).*

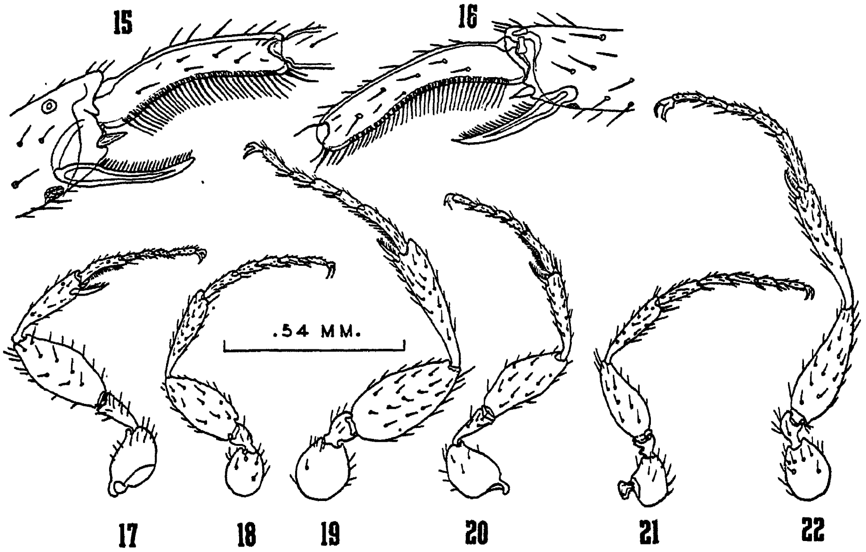
FIG. 12. *Maxillolabial complex (male).*

FIG. 13. *Maxillolabial complex (female).*

FIG. 14. *Mandible (female).*


7
8
.425 MM.
9
10
LBP
PGL
GL
GA
LC
MXP
MT
ST
SMT
CD
11
12
13
14
.082 MM.

The legs of the female and male are very similar in structure (Figs. 15 to 23) the male legs being slightly less robust in form. Both sexes have well developed antenna cleaners on the forelegs (Figs. 15 and 16). The cleaner is formed partly by the proximal tarsal segment and partly by a modified tibial spur or calcar. The proximal tarsal segment is curved, and bears on its concave margin a row of long bristles, which form a comb or strigilis to clean



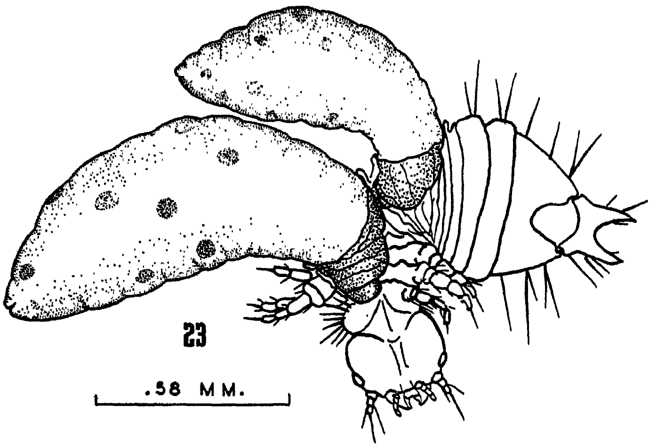


FIG. 15. *Antenna cleaner (female).*

FIG. 16. *Antenna cleaner (male).*

FIGS. 17, 18, 19. *Fore-, mid-, and hind legs of female.*

FIGS. 20, 21, 22. *Fore-, mid-, and hind legs of male.*

FIG. 23. *Two larvae of Cephalonomia waterstoni Gahan, one full grown, the other slightly smaller, feeding on a rusty grain beetle larva.*

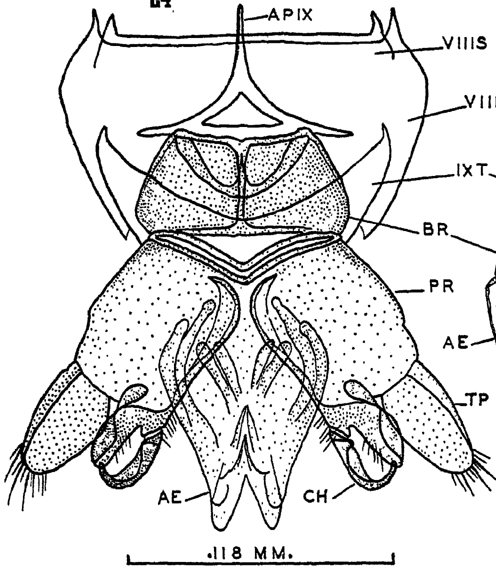
the antenna. The well developed, elongate, pectinate tibial spur is curved outward and then inward, so that its concave side faces the concavity of the strigilis. As a result the antenna is cleaned by the bristles of the strigilis and the calcar when it is drawn between them. An additional small peg-shaped spur is present on the tip of the tibia, between the articulation of the latter with the tarsus and the larger tibial spur. The femur and tibia of the hind legs of both sexes are considerably elongated and the tibiae bear long pectinate spurs at their distal ends (Figs. 19 and 22).

The shiny-black, ovate, nine-segmented abdomen is compressed dorsoventrally. The male copulatory apparatus consists of a pair of clasping organs and an aedeagus. The phallobase, which forms the connection with the ninth segment, is a closed ring or braceletlike structure that articulates with the ninth sternite (Fig. 25). The band is narrower on the ventral side where it has a median sclerotized projection directed anteriorly. The dorsal median part of the band shows a suture where the two ends of the "bracelet" have joined. Fastened to this ringlike sclerotized band are the aedeagus and clasping organs. The aedeagus occupies a median and dorsal position, while the clasping organs are lateral and ventral to it (Figs. 24 and 25). The aedeagus viewed dorsally has the shape of a truncated cone with somewhat concave sides. Its sides show portions rather strongly sclerotized, but in the main the aedeagus appears membranous.

The clasping organs are quite complicated structures; the basal parts of parameres articulate with the phallobase at the proximal end, and distally bear the claspers and tactile plates (Fig. 25). The parameres are oblong structures lying lateral and ventral to the aedeagus. Each bears on the dorsal side at the base a bow-shaped, sclerotized ridge which, when the parameres are closed, lies close to the aedeagus. The inner sides of the parameres, which are associated with the clasping function of these organs, are strongly sclerotized, especially in their distal parts. A row of tactile bristles stands on this more strongly sclerotized area. Articulated with the inner proximal margin of this sclerotized portion is the clasper, a strongly sclerotized hook that curves laterally so that its tip can be made to close upon the apical end of the sclerotized area just described (Fig. 24). To the outside of the clasper is an arched, platelike tactile organ bearing numerous tactile bristles at its tip (Fig. 24). The length of the whole male copulatory apparatus, including the phallobase, is about 0.17 mm. The basal ring or phallobase is about 0.03 mm. wide on the dorsal side and only 0.01 mm. at its narrowest part on the ventral side. The parameres are about 0.12 mm. long, including the tactile plate, which is 0.05 mm. in length.

The female stinging apparatus in *Cephalonomia waterstoni* (Figs. 26 and 27), as in other stinging Hymenoptera, includes not only the usual parts of the ovipositor but also the lateral sclerites (quadrate plates) of the ninth tergite. The quadrate plates, which are somewhat parallelogram-shaped, are strengthened by strongly sclerotized ridges, which at the posterior ends of the plates articulate with the triangular-shaped first valvifers (corner plates). Articulating with the first valvifers are the bow-shaped rami of the first valvulae, which connect distally to the long slender first valvulae or lancets of the sting

24



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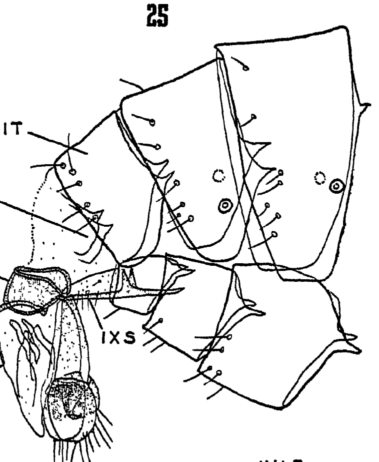

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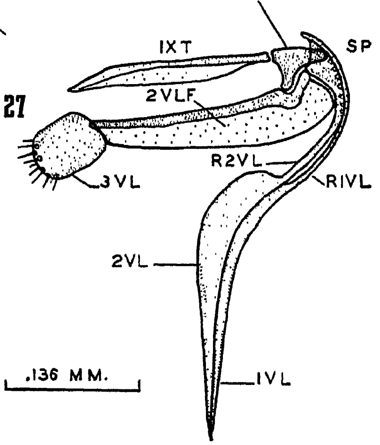

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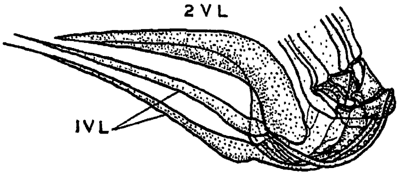

Genitalia of Cephalonomia waterstoni Gahan.

AE: aedeagus; APIX: apodeme of ninth sternite; BR: basal ring; CH: clasping hook; PR: paramere; R1VL: ramus of first valvula; R2VL: ramus of second valvula; SP: sickle-shaped plate; TP: tactile plate; 1VL: first valvula; 2VL: second valvula; 3VL: third valvula; 1VLF: first valvifer; 2VLF: second valvifer; VIIIS: eighth sternite; IXS: ninth sternite; VIIIT: eighth tergite; IXT: ninth tergite.

FIG. 24. *Dorsal view of male genitalia.*

FIG. 25. *Lateral view of male genitalia.*

FIG. 26. *First and second valvulae of stinging apparatus.*

FIG. 27. *Right one-half of stinging apparatus.*

(Figs. 26 and 27). The second valvifers (oblong plates plus sickle-shaped plates) give rise to the rami of the second valvulae, which are connected distally to the second valvulae. The latter unite to form an inverted trough, the median stylet. The slender first valvulae or lancets slide on the lower margins of the median stylet. At the other end of the oblong plates are articulated the third valvulae, which form a sheath in which the first and second valvulae are protected when in repose. The first valvulae are approximately 0.27 mm. in length; the second valvulae are slightly shorter, while the third valvulae are very much shorter. The length of the rami of the first and second valvulae are not included in the above measurements.

The adults apparently are strong fliers, as they have been taken in ventilator traps situated at the apex of the roof of grain bins (3). Schwitzgebel and Walkden in order to determine just what species of insects actually entered grain bins, placed ventilator traps at the apex of the roof of two 1000 bu. steel bins that were tightly caulked so that the only avenue by which insects could gain entrance was through the ventilator traps. Many specimens of *Cephalonomia waterstoni* were taken in these traps.

Sex Ratio of *Cephalonomia waterstoni* Gahan

Since *Cephalonomia waterstoni* males were unknown, an attempt was made to find them in the cultures used in the life history studies. Two hundred live adults were sifted from the grain and placed in a 10% potassium hydroxide solution overnight. Adults treated in this manner could easily be sexed, as the genitalia could then be seen when they were placed on slides and viewed through a microscope. Of the 200 adults examined three proved to be males. From the preponderance of females and the fact that males of the species had not previously been found, it seemed that the male must be of little importance in the life cycle of the insect, and that the species must be mainly parthenogenetic in its habits.

This hypothesis was tested by rearing isolated females so that they were known to be virgins. These unmated females laid eggs, but, when their offspring were sexed, only males were found. This showed that virgin females could only produce eggs that developed into males, or in other words, that unfertilized eggs produce only males. This fact, which disproved the hypothesis advanced above, did not seem to be consistent with the fact that so few males were present in the cultures. It did not seem probable that such a low proportion of males in a species of Hymenoptera with solitary habits would be sufficient to ensure the survival of the species. It then occurred to the writer that possibly the sifting of live adults of *Cephalonomia waterstoni* from a culture that had been established for only a few weeks did not give a true picture of the sex ratio. Therefore the grain from a culture was again sifted, but this time only dead adults were taken and treated with potassium hydroxide. When these adults were sexed only 14 out of a total of 52 were found to be females. It was then perfectly clear that the ratio of females to males was far different from that brought out in the sexing of the initial 200 insects. The fact that females outnumbered males among the live adults, while the opposite was true for the dead adults, could be explained on the supposition that females are longer lived than males. In other words it appeared that in a fairly new culture of *Cephalonomia waterstoni* not many of the females had died while many of the males had succumbed. But this explanation would hardly explain why only three males were found among the 200 live adults sexed, as it would seem probable that more than this number of males would be present in such a large sample.

In order to get a more reliable estimate of the sex ratio 22 mated female parasites were taken and kept isolated in individual rearing cages. Beetle larvae were supplied daily to the female wasps and each day the parasitized larvae were removed and the parasites reared to the adult stage and then sexed. The progeny of each female was kept separate and the number of males and females among a random sample of each female's offspring is given in Table II.

TABLE II

SEX RATIO IN THE OFFSPRING OF 22 FEMALES OF *Cephalonomia waterstoni* GAHAN

Female number	Sexed random sample from offspring of each female		
	Total number sexed	Number of males	Number of females
1	10	3	7
2	1	0	1
3	5	2	3
4	12	4	8
5	6	2	4
6	6	3	3
7	8	2	6
8	15	3	12
9	10	2	8
10	17	6	11
11	6	2	4
12	12	4	8
13	2	0	2
14	5	1	4
15	5	1	4
16	7	4	3
17	1	0	1
18	2	0	2
19	5	2	3
20	9	4	5
21	12	4	8
22	11	3	8
Totals	167	52	115

The data given in Table II suggest that the true ratio of females to males in *Cephalonomia waterstoni* is somewhere near 2 : 1.

Oviposition Rate of *Cephalonomia waterstoni* Gahan

Twenty-five *Cephalonomia waterstoni* females of unknown age were chosen at random from one of the culture jars. Each female was put into a small-size rearing cage that contained three or four fourth instar larvae of *Laemophloeus ferrugineus.* For the next 10 days each female was transferred to a new container containing fresh host larvae. The larvae in each cage, after the female had been removed, were then examined and the number of parasite eggs laid during the preceding 24 hour period was determined. The results of these counts are given in Table III. This table shows that females of *Cephalonomia waterstoni* laid an average of one to three eggs per day, but may lay as many as eight in a single 24-hr. period. No doubt the number of suitable host larvae present has much to do with the rate of oviposition. An attempt was made to provide sufficient suitable host material to obtain the maximum oviposition rate, but three or four host larvae crawling about in an otherwise empty container presented unnatural conditions to the introduced parasite females, which may have influenced their oviposition reactions.

TABLE III

OVIPOSITION RECORD OF 25 *Cephalonomia waterstoni* FEMALES OF UNKNOWN AGE OVER A PERIOD OF 10 DAYS

Insect number	Number of eggs laid on successive days										Total eggs laid in 10 days	Average eggs per female per day
	1	2	3	4	5	6	7	8	9	10		
1	3	4	2	2	2	8	6	0	0	Dead	27	3.0
2	0	1	3	1	0	0	2	2	3	0	12	1.2
3	2	4	3	2	3	4	4	0	0	0	22	2.2
4	1	2	1	0	4	0	0	2	2	2	14	1.4
5	4	1	0	2	4	4	2	4	2	2	25	2.5
6	2	2	2	3	0	2	5	0	1	0	17	1.7
7	4	1	0	1	4	4	0	2	0	0	16	1.6
8	2	2	1	3	2	0	3	0	2	2	15	1.5
9	3	2	2	1	0	1	1	2	0	0	12	1.2
10	2	2	0	3	2	2	4	4	2	1	22	2.2
11	1	5	3	0	4	5	4	2	2	3	29	2.9
12	4	1	2	2	1	3	3	0	0	0	16	1.6
13	4	3	2	1	0	2	4	0	0	0	16	1.6
14	0	2	0	1	0	0	0	0	0	0	3	0.3
15	4	2	4	1	2	5	3	2	1	3	27	2.7
16	2	2	4	1	2	1	4	0	4	2	21	2.1
17	0	2	0	1	1	2	1	1	0	0	8	0.8
18	0	3	1	2	0	4	3	1	0	1	15	1.5
19	0	0	2	1	1	2	3	0	0	1	10	1.0
20	0	1	0	0	0	2	2	0	0	1	6	0.6
21	2	0	0	1	0	0	0	0	0	1	4	0.4
22	1	1	2	4	0	2	2	0	0	0	12	1.2
23	2	4	5	4	2	4	3	0	2	4	30	3.0
24	1	3	1	3	3	5	1	2	2	2	23	2.3
25	–	4	2	2	3	6	4	0	0	2	23	2.6
Totals	44	54	42	42	40	68	64	24	23	24	425	

However, any other method would have made successful egg counts impossible, and the data obtained at least give valid evidence of the potential oviposition rate of *Cephalonomia waterstoni.*

Studies of the Effects of *Cephalonomia waterstoni* Infestations on *Laemophloeus ferrugineus* Populations

Twenty-five adults of *Laemophloeus ferrugineus* were put into each of four different pint mason jars filled with wheat. The jars were then put into an incubator running at 90° F. and 65 to 75% relative humidity. One month later 10 *Cephalonomia waterstoni* adults were put into two of the jars. Forty days after the parasites were introduced the contents of the four jars were screened and the insect counts that were made are given in Table IV. They show that the presence of the parasite had very significantly retarded the increase of rusty grain beetles. This retardation is still more evident when a comparison of the insect populations not removed by screening is made. One hundred kernels of grain chosen at random were dissected and the record of the different stages of *Laemophloeus ferrugineus* found is given in Table V, which also gives the estimated number of insects left per jar after screening.

TABLE IV

NUMBER OF INSECTS SCREENED FROM FOUR TEST JARS USED IN THE FIRST EXPERIMENT TO DETERMINE THE EFFECT OF *C. waterstoni* ON POPULATIONS OF *Laemophloeus ferrugineus*

Jar number	Beetles			Adult parasites	
	Larvae	Pupae	Adults	Alive	Dead
1	3	0	711	163	178
2	4	0	502	156	202
Totals	7	0	1213	319	380
3	257	6	1572	0	0
4	216	4	1209	0	0
Totals	473	10	2781	0	0

Table VI compares the total *Laemophloeus ferrugineus* populations of the four jars. These totals were derived by adding the number of insects actually removed to the estimated totals of those not removed.

It can be seen by the summarized data in Table VI that the introduction of 10 adults of *Cephalonomia waterstoni* into each jar destroyed enough rusty grain beetles in 40 days to reduce their number to approximately one-third of that found in the corresponding control jars. These results were so striking that the experiment was repeated. This time, however, instead of adding 10 parasites to *Laemophloeus ferrugineus* cultures one month old, 25 parasites

TABLE V

NUMBER OF *Laemophloeus ferrugineus* DISSECTED FROM 100 KERNELS OF WHEAT, AND THE ESTIMATED POPULATION LEFT IN THE RESPECTIVE JARS AFTER SCREENING

Jar number	Number of *L. ferrugineus* dissected from 100 kernels of wheat			Estimated number of *L. ferrugineus* left in each jar		
	Larvae	Pupae	Adult	Larvae	Pupae	Adults
1	6	1	3	720	120	360
2	7	0	0	840	0	0
Totals	13	1	3	1560	120	360
3	22	3	3	2640	360	360
4	19	1	2	2280	120	240
Totals	41	4	5	4920	480	600

TABLE VI

TOTAL *Laemophloeus ferrugineus* POPULATIONS OF THE FOUR TEST JARS USED IN THE FIRST EXPERIMENT TO DETERMINE THE EFFECT OF *Cephalonomia waterstoni* ON *Laemophloeus ferrugineus* POPULATIONS

Jar number	Total number of beetles per jar	Jar number	Total number of beetles per jar
Parasites present:		No parasites:	
Jar 1	1914	Jar 3	5195
Jar 2	1346	Jar 4	4069
Total	3260	Total	9264

were added to cultures that were only 13 days old. The insect counts and kernel dissections were made 35 days later instead of 40 days. The results obtained are shown in Tables VII, VIII, and IX. Again the results are very significant, although the reductions obtained in beetle populations were not quite so great as in the first experiment.

These two experiments show that the parasitic wasp *Cephalonomia waterstoni* increases in numbers very rapidly in wheat infested with the rusty grain beetle under laboratory conditions, and that the presence of the parasite causes a marked retardation in the increase of the beetle population. The measure of control that would result from the introduction of this wasp into infested wheat in commercial storage is purely speculative. However, this work definitely shows that this particular parasite can easily be reared in tremendous numbers under laboratory conditions. It also suggests that the introduction of large numbers of the parasite into heavily infested grain, or periodically into infested mills and warehouses, might have far-reaching beneficial effects.

TABLE VII

NUMBER OF INSECTS SCREENED FROM THE FOUR TEST JARS USED IN THE SECOND EXPERIMENT TO DETERMINE THE EFFECT OF *Cephalonomia waterstoni* ON POPULATIONS OF *Laemophloeus ferrugineus*

Jar number	Beetles			Adult parasites	
	Larvae	Pupae	Adult	Alive	Dead
1	0	0	116	132	136
2	1	0	165	103	114
Totals	1	0	281	235	250
3	31	1	356	0	0
4	18	0	363	0	0
Totals	49	1	719	0	0

TABLE VIII

NUMBER OF *Laemophloeus ferrugineus* DISSECTED FROM 100 KERNELS OF WHEAT, AND THE ESTIMATED POPULATION LEFT IN THE RESPECTIVE JARS AFTER SCREENING

Jar number	Number of *L. ferrugineus* dissected from 100 kernels of wheat			Estimated number of *L. ferrugineus* per jar		
	Larvae	Pupae	Adults	Larvae	Pupae	Adults
1	2	0	0	240	0	0
2	13	0	0	1560	0	0
Totals	15	0	0	1800	0	0
3	12	0	1	1440	0	120
4	14	1	0	1680	120	0
Totals	26	1	1	3120	120	120

TABLE IX

TOTAL *Laemophloeus ferrugineus* POPULATIONS OF THE FOUR TEST JARS USED IN THE SECOND EXPERIMENT TO DETERMINE THE EFFECT OF *Cephalonomia waterstoni* ON POPULATIONS OF *Laemophloeus ferrugineus*

Jar number	Total number of insects per jar	Jar number	Total number of insects per jar
Parasites present:		No parasites:	
Jar 1	356	Jar 3	1948
Jar 2	1726	Jar 4	2181
Total	2082	Total	4129

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THE BIOLOGY OF *LAEMOPHLOEUS FERRUGINEUS* (STEPH.)[1]

BY R. OMAR RILETT[2]

Abstract

Life history and morphological studies were made on *Laemophloeus ferrugineus* (Steph.), a coleopterous pest of stored grain and other products.

Temperature greatly affected the rate of development of *L. ferrugineus*. At a relative humidity of 75%, eggs at 80° F. hatched in four to five days, at 90° F. in three to four days, and at 100° F. in two to three days. Similarly, the period from hatching of the egg to the emergence of the imago at 70° F. was from 69 to 103 days, at 80° F. from 26 to 38 days, at 90° F. from 19 to 33 days, and at 100° F. from 17 to 26 days. First instar larvae did not survive at a temperature of 110° F.

An increase in relative humidity within the range from 50 to 75% accelerated development during the larval feeding period. Above 75% relative humidity there was no significant acceleration of development. Mortality was very high at relative humidities below 50%—all larvae failing to develop at a relative humidity of 25%. At 90° F. the period from the hatching of the egg to the emergence of the imago at 50% relative humidity was from 28 to 42 days, at 65% relative humidity from 23 to 33 days, at 75% from 19 to 25 days, at 90% relative humidity from 18 to 25 days, and at 100% relative humidity from 18 to 27 days. The optimum environmental condition for the development of *L. ferrugineus* was from 90° F. to 100° F. at a relative humidity of 75% or higher.

L. ferrugineus populations increased more rapidly in whole rye and wheat grain than in the same materials when they were coarsely ground. Just the opposite was true in the case of oats, barley, corn, sunflower, flax, and soybeans. Whole kernels of rye, wheat, corn, and rice were decreasingly susceptible to injury in the order named, while whole kernels of oats and barley, and the seeds of sunflower, flax, and soybeans were practically free from injury by *L. ferrugineus* at humidity levels normally occurring in stored grains and seeds. Although first instar larvae did gain access to the wheat germ of grain described commercially as being "whole, sound, and undamaged", they were unable to successfully attack whole wheat kernels that had no breaks in their bran layers. The breaks need only to be microscopic in size to allow penetration by the larvae.

Introduction

The rusty grain beetle, *Laemophloeus ferrugineus* (Steph.), formerly known as the rust-red grain beetle, belongs to the family Cucujidae and was described by Stephens about 1845. Leng (6) listed *Cryptolestes* as a subgenus of *Laemophloeus* and designated the insect as *Laemophloeus ferrugineus* (Steph.). Casey (4), on the other hand, maintained that *Cryptolestes* is a very distinct and well characterized genus, and Sheppard (10) appears to agree with Casey, since he designated the insect as *Cryptolestes ferrugineus* Steph. In the literature, however, Leng's classification is most often used, and the insect is referred to as *Laemophloeus ferrugineus* (Steph.).

Leng listed *ferrugineus* (Steph.), *testaceus* (Payk.), and *monilicornis* (Steph.) as synonymous specific names for this species.

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This work, which was instigated by Dr. H. E. Gray, In Charge, Stored Product Insect Investigations, Ottawa, and Dr. B. N. Smallman, Entomologist, Board of Grain Commissioners, for Canada, was begun at the University of Western Ontario, London, Ont. and completed at the University of Wisconsin, Madison, Wis. The work at the latter university was supported in part by the Wisconsin Alumni Research Foundation.

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A comprehensive survey of the literature shows that very little has been reported about the biology of the rusty grain beetle, although many references to its presence and importance exist. Casey (4) and Wheeler (12) gave its distribution as cosmopolitan and the literature certainly gives ample supporting evidence for this statement. Only two references to its biology were found: Belyaev, Shesterikova, and Popov (2) and Sheppard, E. H. (10). The first article deals with the insect as a pest of oil seeds in Russia, while the second is concerned with the biology of the insect in relation to *Trichogramma minutum* parasite rearing work. Sheppard's attention was attracted to the beetle when it became established in the incubators used for rearing the Angoumois grain moth at Colorado State College. It fed upon the eggs of the grain moth that were used in rearing *Trichogramma minutum*, a parasite of codling moth eggs.

In the United States the damage to stored grains by primary grain pests such as the granary weevil, *Sitophilus granarius* (Linn.), the Angoumois grain moth, *Sitotroga cerealella* (Oliv.), the rice weevil, *Sitophilus oryza* (Linn.), and the lesser grain borer, *Rhyzopertha dominica* (Fab.), is so tremendous that the injury caused by *Laemophloeus ferrugineus* is more or less overlooked. For example, no specific reference was found on its occurrence in Wisconsin, yet upon investigation it was found in goodly numbers in the University of Wisconsin's feed storage building, and therefore it is likely prevalent throughout the state. It has been reported from most of the 48 states, however, and Farrar and Flint (5) report it widespread in stored corn in Illinois. A recent survey carried out in California by Linsley and Michelbacher (7) showed that *Laemophloeus ferrugineus* was present in 3 out of 42 insect-infested granaries examined in the Sacramento Valley; 9 out of 37 in the San Joaquin Valley; 7 out of 33 in the Coastal Region; and 5 out of 16 in the Southern Interior. These workers listed *Laemophloeus ferrugineus* among the secondary pests of grain. It was their belief that this insect rarely attacks whole grain at the moisture level that prevails in normal storage, but that it is associated with the primary pests and follows closely upon their attack. Although this may be true for California conditions it does not hold for the entire continent, as will be brought out later.

A severe outbreak of granary pests occurred in flour mills in the Russian Union in 1937 and a survey was carried out in July of that year in Moscow and the province of Voronezh (11). Of the pests found, *Laemophloeus testaceus* Fab., *Laemophloeus ferrugineus* (Steph.), and *Tribolium confusum* Duv., were the most injurious and were abundant in all the mills examined. The first two had previously been considered as unimportant and restricted to the south of the Russian Union. In the mills they occurred in tunnels in crusts of flour that formed on the walls of certain parts of the equipment as a result of the contact of the warm flour with the cold surface of the walls and the condensation of moisture there. *T. confusum* infested thicker crusts than those in which *Laemophloeus* occurred.

In normal times Canadian grains move forward steadily, allowing little opportunity for insects to become established, and, in the western provinces particularly, the severe winter temperatures have kept insect infestation of stored grain practically negligible. Yet between 1939 and 1944 the rusty grain beetle became a major grain pest in Canada, and at that time ranked as the most important insect pest of stored grain in Western Canada (3).

The phenomenal increase in rusty grain beetle infestations in Western Canada was intimately associated with the temporary storage of wheat during World War II. The types of storage used and typical cases of *Laemophloeus ferrugineus* infestations were decribed by Dr. B. N. Smallman in a paper entitled, *Entomological Aspects of Wartime Grain Storage* in the May 1945 issue of *Grain.*

General Methods

Small, seamless, metal, $\frac{1}{4}$ oz. salve boxes, with a $\frac{1}{2}$ in. hole punched in the lid were used for individual rearing cages. The hole in the lid was covered with silk bolting cloth, held in place by Duco household cement. These boxes proved very satisfactory for rearing and examining the larvae. Each box was numbered to prevent errors that might have occurred if the lids had been numbered and then accidently interchanged.

Two larger sizes of metal rearing cages (3 oz. and 6 oz.) were used in some of the experiments. The cages were arranged on wire mesh trays that could easily be removed from a metal rack on which they were supported while in the incubator. In those experiments where a definite constant humidity was maintained, the cages were supported by three-tiered metal trays that were built to fit into desiccators (9).

Thermostatically controlled incubators, provided with electric fans to keep the warmer air from becoming stratified at the top of the chambers, were used. Temperatures within one Fahrenheit degree of that desired were maintained.

A fairly uniform relative humidity was provided in each incubator by two jars containing water and provided with cheesecloth wicks extending up to the bottom shelf of the incubator.

Eggs or adults for the various experiments were obtained by screening heavily infested, moldy wheat through a four-layered, brass frame, 5 in. sieve set. The sieves, starting at the bottom, were of a mesh to give 80, 60, 40, and 20 openings per linear inch respectively, in this order. The grain remained on the top screen, the adults collected on the 40-mesh screen, and the eggs on the bottom screen. The 60-mesh screen was included to remove some of the broken particles of wheat that were small enough to come through the coarser sieves. Finer debris, and mites when present, passed on through the 80-mesh screen to the bottom and were destroyed after each screening. This procedure prevented the accumulation of debris in the culture, and at the same time provided a very efficient method for the control of mites.

No natural enemy of the beetle became troublesome with the exception of the common yellow house ant (*Monomorium pharaonis*). While some renovation work was being carried out in the laboratory the cultures were not inspected closely for a period of a few weeks until it was noticed that ants had invaded the incubators, eaten their way through the bolting cloth into the jars, and were carrying off the larvae of *Laemophloeus ferrugineus* at a rapid rate. Several thousand ants were present and a procession of ants, each ant with a larva grasped between its mandibles, was observed emerging from one of the culture jars.

They were quickly brought under control by thoroughly cleaning out the incubator and painting its legs, floor, and walls with a solution of D.D.T. in oil. Tree Tanglefoot was smeared around the openings in the lids of the jars and cages. These measures brought the ants completely under control, and they gave no further trouble.

In life history studies and in experiments where the time of each molt was to be recorded, a single thick cross section of a wheat kernel through the germ region was used as food in each rearing cage. This food was chosen for the following reasons: (1). Previous observations had shown that the larvae had a strong preference for the embryo of wheat. (2). No opportunity was provided for the larvae to burrow into the food and thus become hidden from view. (3). Larvae fed on wheat germ developed more rapidly and more uniformly than those fed on any other portion of wheat, or even on whole wheat. (4). The larvae showed no inclination to wander about when provided with this food. (5). Survival was practically 100% when wheat germ was provided. (6). Sometimes wheat germs are found that are relatively dark in color. These seem to be less favorable as larval food, for development is noticeably retarded when they are fed. The use of cross sections facilitates the detection of these undesirable germs and permits their immediate replacement by another cross section. (7). The cast exuviae were easy to find, which was not the case when meal, flour, or whole wheat was used as food.

A very simple method for the accurate sexing of live beetles was devised. The beetles to be sexed were put in a rearing cage and the cage with its lid removed was placed on finely chopped ice held in a Petri dish. As soon as the floor of the cage became chilled, the beetles became inactive and completely helpless. They could then be studied under the binocular microscope even more satisfactorily than mounted specimens, as the legs, wings, etc., could be moved about by small needles without any danger of breaking them off. The males and females were determined by characteristic differences in the appearance of their antennae, mandibles, and genitalia. In the few instances where doubt remained as to the correct sex, it was necessary to apply light pressure to the ventral part of the abdomen with a pair of very small blunt forceps. If the beetle were a female the "styli" and "substitutional ovipositor" were extruded as shown in Fig. 10. If carefully carried out, this procedure did not harm the beetle.

Life History of the Rusty Grain Beetle, *Laemophloeus ferrugineus* (Steph.) at 90° F. and 75% Relative Humidity

Eggs are deposited by *Laemophloeus ferrugineus* females in small crevices, furrows, fractures, or holes in the wheat kernels, in spaces between the kernels, or among the detritus, by means of the "substitutional ovipositor" (Fig. 10), consisting of the telescoped caudal segments of the abdomen. During oviposition these segments, which normally are retracted within the abdomen, are protruded posteriorly and extended deep into the groove in which the egg is placed. The terminal segment bears a pair of "styli", which probably act as manipulators, helping to orient the egg into the crevice. Eggs have been found, upon dissection of the wheat kernel, in the germ region just under the outer layers of the seed coat, showing that they had been placed there by the insertion of the "substitutional ovipositor", through the small opening often present in the germ end of the wheat kernel. Many eggs are found in the spaces between the individual kernels of grain.

The eggs hatch in three to four days after oviposition. Just prior to the emergence of the larva its segmentation is dimly perceptible through the chorion. A series of undulating movements, caudocephalad, are set up by the larva, and the chorion is broken by the pressure of the larva's head capsule on one end of the egg. The larva continues this activity until its head emerges. It crawls out head first, pulling with its legs and at the same time bending backward and forward, until its attempt to free itself from the shell is successful. The empty egg shell has a very pronounced iridescence. Occasionally it adheres to the larva for some time, being drawn along with the larva as it goes in search of food.

Soon after hatching the larva sets out in search of suitable food. It shows a marked avidity for the wheat germ and if this portion of the wheat is available the larva will invariably be found feeding on it. There is sufficient food in the germ of one wheat kernel to carry the larva through its developmental stages at 90° F. and 75% relative humidity, but at lower humidities or at lower temperatures the amount of food consumed is greater. The larvae also feed on the endosperm, especially in heavily infested grain that has been extensively degermed by earlier generations of the insect. In such cases the entire kernel may become hollowed out, leaving only the outer seed coats as an empty shell.

The larva molts four times, the last molt revealing the pupa (Figs. 28 to 36). The first larval stadium varies from three to four days, about 65% of the first instar larvae having a stadium of four days. The second stadium varies from two to five days, about 45% of the second instar larvae having a stadium of two days and about the same percentage having a stadium of three days. The third stadium is about the same length as the second, while the fourth stadium is much longer, the last two days being spent in an inactive prepupal stage. This stadium varies from five to eight days, the usual period being six or seven days.

During larval development, if the food supply is satisfactory, the larva remains within its burrow, which becomes larger as the wheat germ is consumed. The fecal material and molted exuviae are pushed out of the excavation through the opening used by the larva in gaining access to the wheat germ or that through which the egg was deposited. Larvae may sometimes leave their burrows and wander to other kernels of grain in search of more favorable food. The helpless prepupal or pupal stage is often attacked and devoured by a younger relative that enters its burrow. Young larvae feeding on prepupae and pupae have often been observed after the removal of the seed coat of a kernel of wheat so as to expose the larval burrow.

In a wheat grain habitat the larvae normally pupate within the cells that are left after the consumption of the wheat germ. Before entering the quiescent prepupal stage they effectively close the openings of their burrows with excrement and debris sealed together with silken threads. The caudal end of the larva then becomes stuck to some part of the burrow by an exudation from the anus and the anterior portion becomes more or less supported in a hammock of silk threads that are laid down during the lateral swaying of the anterior end of the body, its caudal end remaining anchored. This hammock is very simple and consists of only a few threads that serve to hold the prepupa in place. Some larvae pupate in the spaces between the kernels of grain and in hollowed-out spaces in the grain detritus.

If only a thin cross section of wheat is used as food in the rearing containers, the full-grown larvae crawl restlessly about in search of suitable places to pupate. However, if a little earth is added to the container, each larva hollows out a small cell and pupates as it would in grain. If a few bran flakes are added it cements some of these together to form a dome under which it enters the prepupal stage.

The pupa, with the dorsal portion of the prothorax appearing first, emerges from the last larval exuviae through the anterior dorsal region of the old larval exoskeleton, leaving the old head capsule attached to the rest of the exuviae on the ventral side. Then by a series of expansions and contractions of the emerging pupa's thorax and abdomen, and by a series of backward and forward movements of the abdominal segments, the exuviae is shunted caudad. The molting process is facilitated by molting fluid. During molting and immediately afterward the abdomen shortens and becomes more or less triangular in shape. The pupal period lasts about four days. On the third day the white pupa changes to a light tan, the mandibles becoming colored first.

When the adult emerges, the old integument of the pupa is sloughed off in much the same way as in the ecdysis of the larva. After the legs and wings break free, the very thin, transparent exuviae is shoved caudad by the legs and the shunting movements of the abdomen. The last segments, which later are telescoped into the abdomen, are also protruded to aid in casting off the exuviae. Shortly after emergence the second pair of wings are extended for a time and then folded beneath the elytra. The adult is of a light tan color when it emerges but changes to a rusty brown within a day or two.

Adults mate in one or two days after emergence and oviposition starts shortly thereafter. Eggs have been observed that were laid by females only two days old. When the male recognizes a female that is physiologically ready to mate, he turns and follows her. He rapidly overtakes her and follows along with his head touching the tip of her abdomen until she stops. He then strokes her elytra with his antennae and nudges her along the sides and end of the abdomen with his head; sometimes he crawls upon her back as well. Then he turns around and backs up, often backing in the wrong direction so as to miss her entirely or to come in contact with the side of her body or even against her head. During all this the female remains quiet; the only movements noticeable are the flickering of her antennae. The male persists in his trials to contact the end of her abdomen, and when he succeeds, shoves strongly backward. In a few seconds they become very tightly coupled together, the ends of the abdomens touching with the tips of the male's elytra usually resting upon those of the female. One pair kept under observation for a considerable period of time was observed to remain together in copulation for 105 min. after which they became separated for 20 min. They then became coupled for a second period of 35 min. This was followed by a third copulation period of 95 min. This same pair were observed copulating again the following day. Other copulating pairs that have been observed showed similar mating habits.

The adults feed on damaged kernels and wheat dust. After an infestation has been established the favorite feeding sites are the burrows in which the larvae have developed and from which the adults have eaten their way to freedom. They return to these burrows when hungry and feed on the remaining portions of germ or endosperm.

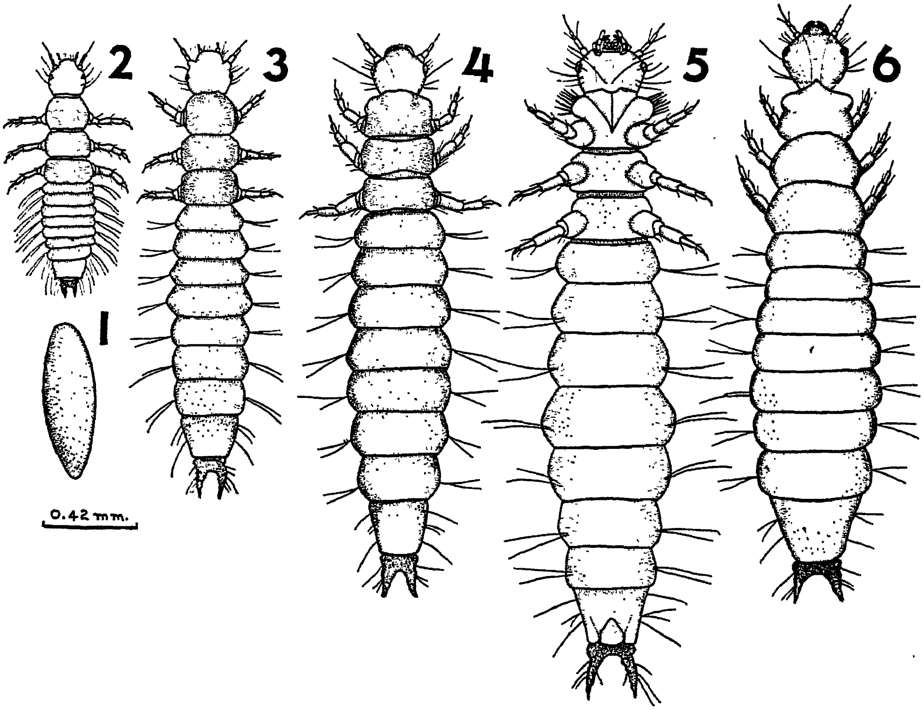
Life Cycle Stages of *Laemophloeus ferrugineus* (Steph.)

The Egg (Fig. 1 and Fig. 38)

The eggs are quite large in relation to the size of the insects that lay them and when isolated on a clean dark surface are quite easily seen with the naked eye. They are about three and one-half times longer than wide with one end slightly more pointed than the other. The dimensions of 10 typical eggs are given in Table I.

The eggs measured varied in length from 0.68 mm. to 0.81 mm., giving an arithmetical average length of 0.76 mm., while the variation in width was from 0.20 mm. to 0.30 mm., with an arithmetical average of 0.23 mm. Occasionally long thin eggs or short wide ones were encountered that did not fall within the range of those measured.

The eggs are white in color and moderately translucent, the embryo being dimly visible through the chorion prior to eclosion. When the eggs are first laid the chorion is easily broken, even when carefully handled with a squirrel's hair brush. The empty egg shell has a very pronounced iridescence. Occasionally it adheres to the larva for some time, being drawn along with the larva as it goes in search of food.



Egg and larval instars of Laemophloeus ferrugineus (Steph.)

FIG. 1. *Egg.*
FIG. 2. *First instar, larva one day old; dorsal view.*
FIG. 3. *Second instar, larva eight days old; dorsal view.*
FIG. 4. *Third instar, larva 12 days old; dorsal view.*
FIG. 5. *Fourth instar, larva 18 days old; ventral view.*
FIG. 6. *Fourth instar, larva 20 days old (prepupa); dorsal view.*

TABLE I

MEASUREMENTS OF 10 TYPICAL EGGS OF *Laemophloeus ferrugineus* (STEPH.)

Egg number	Length in mm.	Width at widest point in mm.
1	0.78	0.20
2	0.78	0.20
3	0.71	0.30
4	0.78	0.23
5	0.78	0.20
6	0.78	0.20
7	0.75	0.23
8	0.68	0.27
9	0.81	0.20
10	0.78	0.23
Average	0.76	0.23

The Larva (Figs. 2 to 6)

The newly emerged, crystal white larva is slightly longer than the egg. The head capsule and caudal hooks very soon become light tan in color. There are four instars and a prepupal stage.

The fully grown larva is roundish, robust, and slightly compressed dorsoventrally when well fed, but greatly flattened if undernourished. The comparative sizes of the four instars are shown by Figs. 2 to 6, inclusive. The head and thoracic segments of the fourth instar are narrower and shorter than the segments of the abdomen, which become progressively wider posteriorly until the fourth segment, then gradually taper caudad to the terminal segment, which bears the caudal hooks. These hooks are very efficient ambulatory aids in the backward progression of the larva in burrows or crevices. The abdomen of the larva is first lengthened so as to extend the caudal hooks back farther in the crevice. Then the hooks are bent dorsad and their pointed tips anchored into a groove or onto a projection in the crevice. The abdomen is then shortened and at the same time the hooks are bent still farther dorsad. Both of these movements cause the larva to be pulled in a backward direction. The larva may also push backward with the three pairs of well developed thoracic legs in order to aid in this movement.

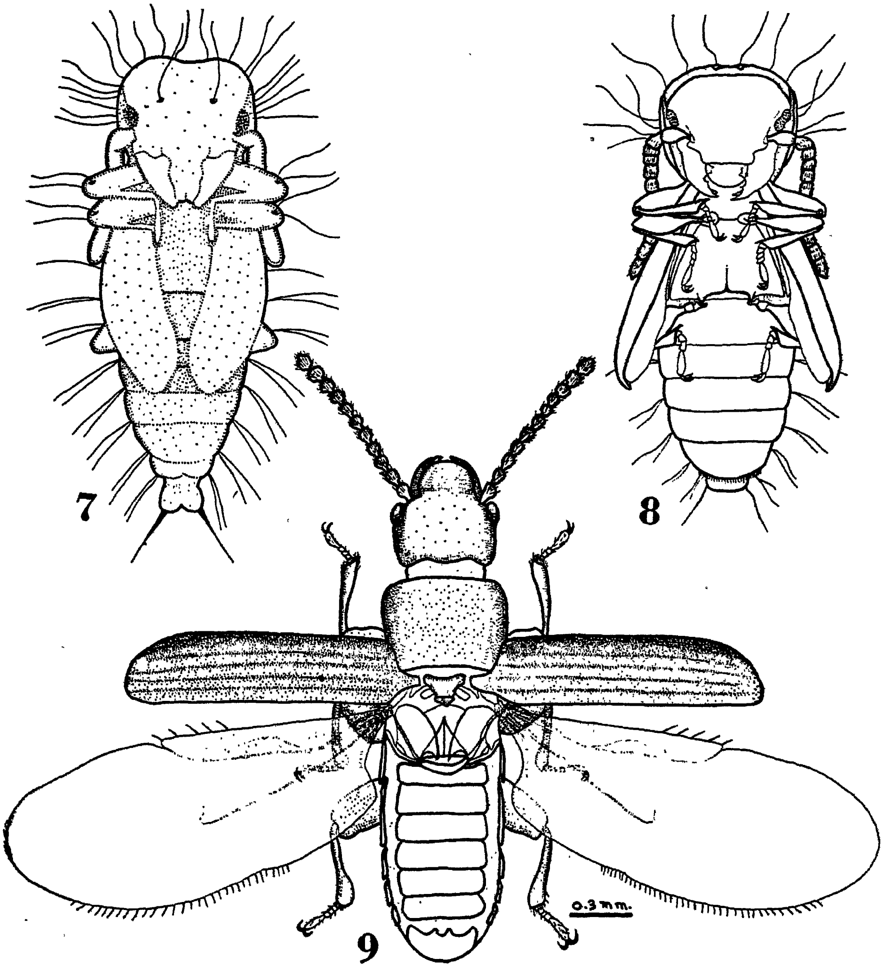
The mouth parts of the larva are very similar to those of the adult (Figs. 14 to 20), as might be expected from the similarity of their feeding habits.

On the anterior lateral margin of the prothoracic segment are two papillae, one on each side. Each papilla, which is first noticeable in the third instar and very pronounced in the fourth, is crowned by a tuft of whitish spines from which silken threads seem to originate. Silken threads appear to issue from both papillae simultaneously, not in sufficient quantity to spin a cocoon but only enough to form a network, somewhat spider-web-like, which helps hold together flakes of bran, debris, fecal pellets, etc., in a makeshift shelter.

The Pupa (Figs. 7 and 8)

The young pupa is white in color, with the exception of the compound eyes, which appear as dark brown spots on each side of the head. The mandibles soon become light tan in color, and about the third day after pupation (at 90° F.) the whole pupa begins to change to a light tan, gradually becoming darker until the emergence of the beetle. At 80° F. this color transition takes place from the fourth to the sixth day after pupation. The pupa is enclosed in a thin transparent integument, which is molted at the time the adult emerges.

The shape of the pupa is somewhat triangular. The future adult head lies ventrad to the prothorax, with the mouth parts directed caudad. Legs, wings, and antennae are folded neatly against the body. The wings originate dorsally, but extend laterally and ventrally so that they cover a large portion of the ventral surface of the pupa, leaving the two pairs of forelegs uncovered while covering the hind pair. The antennae extend laterally from the area



Pupal and adult stages of Laemophloeus ferrugineus (Steph.)
FIG. 7. *Pupa, two days old; ventral view.*
FIG. 8. *Pupa, six days old; ventral view.*
FIG. 9. *Dorsal view of female, with wings extended.*

between the eyes and the base of the mandibles, then dorsally and posteriorly, lying close to the wings, just dorsal to the forelegs.

The Adult (Figs. 9 and 23)

When the beetle first emerges the head, antennae, thorax, and legs are light amber in color and the mandibles reddish-brown. The ventral segments of the abdomen are also a light amber with the conjunctivae colorless, as are all the other conjunctivae of the body. The elytra are translucent white and the flying wings a transparent white, the former turning to reddish-brown within a few hours.

A condensed description of the adult is as follows: Beetles depressed, elongate; antennae 11, segmented, elongate, last three segments slightly enlarged, nearly as long as elytra; scutellum distinct; elytra rounded at tip and covering the abdomen, emarginate, striate, punctate, slightly longer than head and thorax together; front coxal cavities widely separated; anterior coxal cavities broadly and completely enclosed by the sterna, merely a fine suture between each side piece and the median lobe; front coxae rounded, not prominent; hind coxae widely separated, transverse; abdomen with five free ventral segments, unequal in length, the first segment being longer and apparently the result of a fusion of the embryonic first and second abdominal sternites; legs rather short; femur large; tibia slender, terminated by two spurs; tarsi five-segmented with the exception of the male hind tarsi, which are four-segmented; head and thorax finely punctured, the latter narrowed behind, sides curved sinuate near hind angles, which are sharp and prominent.

Blatchley in his key to the Coleoptera of Indiana described the males of the genus *Laemophloeus* as having the middle and hind tarsi four-segmented, while Sheppard (10) stated that the tarsi of both the males and females of *ferrugineus* are all four-segmented. Upon careful examination both these statements appear to be incorrect, the tarsal formula of the female being 5-5-5 and of the male 5-5-4 (Figs. 24 to 27). Furthermore, Sheppard described the hind coxae as "nearly contiguous, transverse", which is not descriptive of the

Morphology of Laemophloeus ferrugineus (Steph.)

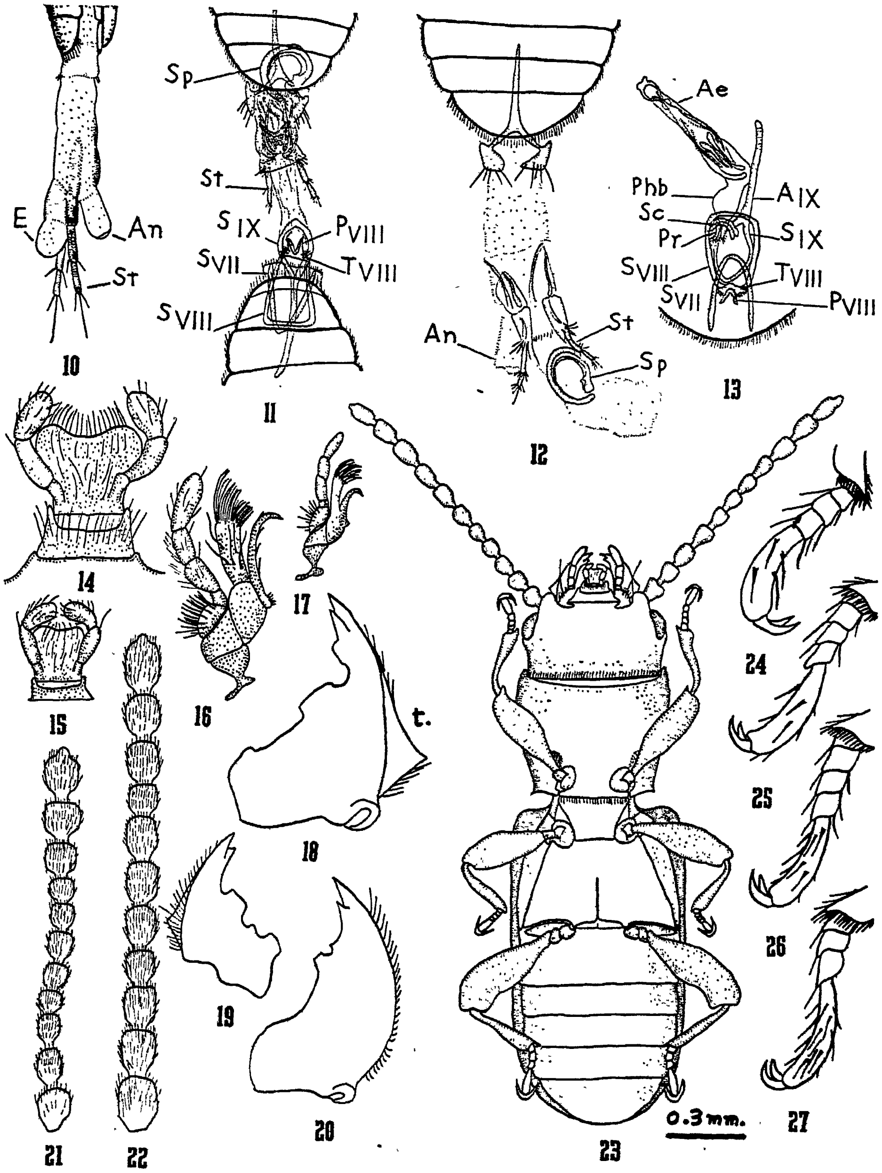
FIGS. 10 to 13. *Genitalia of Laemophloeus ferrugineus.*

Ae: aedeagus; An: anus; AIX: apodeme on sclerotization of the ninth abdominal segment; E: egg exit and female genital opening; Phb: phallobase; Pr: parameres; PVIII: process of eighth tergite; Sc: sclerotization (possibly in the walls of the genital chamber); Sp: sclerotization of spermatheca; St: stylus; SVII: sclerotization of seventh abdominal segment; SVIII: sclerotization of eighth abdominal segment; SIX: sclerotization of ninth abdominal segment; TVIII: tergite of eighth abdominal segment.

FIG. 10. *Caudal end of female with genitalia extended (lateral view).*
FIG. 11. *Genitalia of a copulating pair of beetles (beetles have been pulled apart slightly).*
FIG. 12. *Female genitalia extruded from the abdomen.*
FIG. 13. *Male genitalia within abdomen (aedeagus is bent out of natural position).*
FIGS. 14-20. *Mouth parts of Laemophloeus ferrugineus; t = toothlike projection.*
FIG. 14. *Labium, adult.*
FIG. 15. *Labium, fourth instar.*
FIG. 16. *Maxilla, adult.*
FIG. 17. *Maxilla, fourth instar.*
FIG. 18. *Mandible, adult (male).*
FIG. 19. *Mandible, fourth instar (male).*
FIG. 20. *Mandible, adult (female).*
FIG. 21. *Antenna, female.*
FIG. 22. *Antenna, male.*
FIG. 23. *Adult male, ventral view.*
FIG. 24. *Middle tarsus, male; five-segmented.*
FIG. 25. *Front tarsus, male; five-segmented.*
FIG. 26. *Hind tarsus, female; five-segmented.*
FIG. 27. *Hind tarsus, male; four-segmented.*

strain used in these investigations, as the hind coxae were actually more widely separated than the anterior ones, although they were decidedly transverse (Fig. 23).

The male mandible (Fig. 18) differs greatly from that of the female (Fig. 20), the former having a toothlike projection (Fig. 18, *t*) on the lateral ventral side near the base. The male's antennae are, on the average, longer than the female's, with the terminal three segments relatively not so enlarged (Figs. 21



and 22). The thorax tends to be more contracted behind, and its maximum width relatively greater in the male; also the head of the male tends to be somewhat larger than that of the female.

In order to determine the proportion of males to females in a normal adult population, 125 beetles were sexed. Of these, 48 proved to be males and 75 females, or 61.6% females and 38.4% males. The percentage of females from this count is not quite as high as that found by Sheppard who reported that 75% of adults are females.

The male phallus comprises a single median intromittent organ of tubular form (Fig. 13). The ninth segment of the abdomen retracts into the eighth, and the eighth into the seventh (Fig. 13). During coition these segments, which are terminated by the tubular aedeagus, are protruded, the intromittent organ being inserted deeply into the genital chamber of the female (Fig. 11).

A copulating pair in a rearing cage may readily be studied if the cage is placed on ice. Then with two small forceps the beetles may be pulled apart very slowly while observations are being made through the binocular microscope. As the adults are pulled apart, the female and male genitalia are drawn from within the female's genital chamber. The aedeagus may be seen inserted deep into the female's genital tract, which explains why the two beetles are so firmly attached to one another during coition (Fig. 11).

Styli are present on the ninth abdominal segment of the female (Figs. 12, 11, and 10) but are never present in the male.

Flight Habits of *Laemophloeus ferrugineus*

Sheppard reported that adults, when they attempted flight, were unable to clear the rim of a Petri dish that was one-half inch in height. This is contrary to observations made during the present investigations, as the writer has watched adults fly out of the metal rearing cages, which required a minimum takeoff angle of 25° in order to clear the rim of the cage. This is a greater angle than would be necessary to clear the rim of the Petri dish described by Sheppard. On Aug. 8, 1944, two beetles were observed to fly up to a height of 5 ft. above the window sill in a laboratory room where the insect was being studied. The temperature at the time was 86° F. The beetles were observed during the whole flight and they rose almost vertically for the whole distance. It is therefore quite evident that they are relatively good fliers when the temperature is high. This conclusion agrees with many succeeding observations that show, however, that at temperatures of 70° F. or below the beetle is relatively inactive.

While considering the flight habits of this beetle it is interesting to note that Barnes and Kaloostian (1) studied the flight habits of certain insects that infest stored raisins at Fresno, Calif. These workers found that the group *Laemophloeus* spp., which probably consisted for the most part of *ferrugineus* (Steph.) (which has frequently been found associated with stored raisins), was most abundant in hot weather—July and August being the months of greatest activity. They reported a small amount of flight throughout the day when the temperature was above 65° F., with a period of increased activity for about two hours late in the afternoon, reaching a maximum from about 30 min. before sunset to 15 min. after.

Oviposition Rates of *Laemophloeus ferrugineus* at 90° F. and 65 to 75% Relative Humidity

No method was devised for determining exactly the number of eggs laid per day by a female of the rusty grain beetle, but an experiment was carried out that gave the total adult descendants of 20 individual females over a period of 23 days. Thus the approximate number of eggs laid per day could be estimated.

Twenty pairs of beetles were obtained while they were united in copulation. Ten pairs, one pair to each cage, were put into 3-oz. rearing cages. The other 10 copulating pairs were left undisturbed until they had finished mating and then the females, one to each cage, were put into similar cages. All of the cages had previously been filled with whole wheat.

By the above procedure it was certain that 20 mated females had been obtained, and that 10 of the cages each contained an isolated, inseminated female, while the other 10 each contained a male and female.

The cages were put into an incubator at 90° F. and 65 to 75% relative humidity and left for 46 days, when the adults present were counted. By choosing exactly 46 days the offspring of only the original female would be counted because the first of the third generation adults would not appear until about the 47th or 48th day under the conditions of the experiment. However, two possible sources of error remain: (1) the unknown mortality of the larvae, and (2) the unrecorded eggs that failed to hatch. Observations made during the course of other investigations lead to the belief that the mortality rate of uncrowded populations provided with suitable food is relatively low. It had also been noted that the percentage of eggs that hatch is quite high. Thus the progeny counts should give at least a good indication of the number of eggs laid per day by the individual females.

The results of this experiment are given in Table II. Five hundred and eighty-four eggs were laid by the 10 females living with males, or an average of 2.54 eggs per day per female, while the 10 isolated females laid 414 eggs or an average of 1.80 per female per day. This would indicate either that the females were stimulated to oviposition by periodic copulation, or that isolated females become infertile. Further experimentation would be necessary to establish final conclusion on this point. However, we may safely say that females of *Laemophloeus ferrugineus* can lay at least two or three eggs per day in whole grain at a temperature of 90° F. and a relative humidity of 65 to 75%.

TABLE II

TOTAL ADULT DESCENDANTS OF INDIVIDUAL *Laemophloeus ferrugineus* FEMALES FROM AN OVIPOSITION PERIOD OF 23 DAYS

Insect number	No. of beetles at end of experiment	No. of beetles at start of experiment	Increase	Average number of eggs laid per day
Pair 1	67	2	65	2.83
“ 2	52	2	50	2.17
“ 3	91	2	89	3.87
“ 4	68	2	66	2.87
“ 5	49	2	47	2.04
“ 6	39	2	37	1.61
“ 7	56	2	54	2.35
“ 8	71	2	69	3.00
“ 9	55	2	53	2.30
“ 10	56	2	54	2.35
	Average number of eggs laid per day per female			2.54
Female 1	37	1	36	1.57
“ 2	57	1	56	2.43
“ 3	30	1	29	1.26
“ 4	43	1	42	1.83
“ 5	61	1	60	2.61
“ 6	63	1	62	2.69
“ 7	42	1	41	1.78
“ 8	37	1	36	1.57
“ 9	22	1	21	0.91
“ 10	32	1	31	1.35
	Average number of eggs laid per day per female			1.80

Temperature–Humidity Studies in the Development of *Laemophloeus ferrugineus* (Steph.)

EFFECT OF RELATIVE HUMIDITY ON THE LENGTH OF THE LARVAL AND PUPAL STAGES OF *Laemophloeus ferrugineus*

Desiccators were used as constant humidity chambers (9). Sulphuric acid solutions of definite strength prepared in the proportions shown in Table III were used to obtain the desired humidities (13).

TABLE III

SULPHURIC ACID SOLUTIONS USED IN CONSTANT HUMIDITY CHAMBERS

Solution	Relative humidity, %	Amount of 95.5% sulphuric acid, cc.	Amount of distilled water, cc.	Specific gravity of solution
A	10	368	321	1.55
B	25	318	415	1.45
C	50	247	545	1.33
D	65	205	622	1.27
E	75	173	682	1.22
F	90	105	806	1.14

Solution A was prepared by adding 368 cc. of sulphuric acid to 321 cc. of distilled water. The acid was added very slowly to the water and the resulting solution allowed to stand until it cooled to room temperature. The specific gravity of the solution was then determined with a hydrometer, and either more water or more acid was added, if necessary, to bring the specific gravity of the solution to exactly 1.55. Each of the other solutions (B, C, D, E, and F) was prepared in a similar manner, using the amounts of acid and water given in Table III and then adjusting to the correct specific gravity.

Although these solutions did not provide the exact humidity desired at the different temperatures, they did give very close approximations because the variation in the percentage of sulphuric acid needed to give the same humidity at different temperatures is very slight if the temperature changes are not great.

An exploratory experiment was carried out to study the effect of relative humidity on development at temperatures of 80° and 90° F. The corners of a square piece of wire netting were bent down to form "legs" and the resulting wire "table," which fitted snugly into the desiccator, was placed so that its "legs" rested on the inner ledge of the desiccator. On this "table" was placed a wooden slide box, with glass slides in every second pair of grooves. Glass vials were stood between the rows of slides. Each box held 35 of these vials. A grain of wheat split longitudinally through the middle was put into each vial. Each of the 11 remaining desiccators was provided with a similarly prepared battery of rearing vials.

The desiccators were placed in the proper incubators running at constant temperatures of 80° and 90° F. After eight days, which allowed time for the moisture content of the wheat and the air in the desiccator to reach or approach equilibrium, eggs, one to each vial, were added to one of the batteries. On succeeding days vials in the remaining batteries were supplied with eggs. Each vial was provided with a cotton stopper, after being supplied with an egg.

The split kernel of wheat in each vial exposed to 90% relative humidity was covered with a dense growth of mold when the eggs were introduced, and this growth remained luxuriant during the period of the experiment.

The vials were examined each day until the eggs had hatched, or until it was definitely ascertained that they would not hatch. The battery was then left undisturbed until the pupae began to appear, when the vials were once again examined daily to note the date of emergence of adults. It will be noted in Table IV that in the battery kept at 75% relative humidity and 90° F. the adults had all emerged before the first examination was made. This was due to the fact that the interim between the hatching of the eggs and the emergence of the adult was shorter than had been anticipated. The average period from hatching to adult in this case was 20 days or less. In another experiment where 20 adults were reared under the same conditions of humidity and temperature but examined daily, this period averaged 20.5 days, so it is unlikely that the correct average in the first experiment would be much less than the 20 days recorded.

TABLE IV

EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON THE RATE OF DEVELOPMENT OF *Laemophloeus ferrugineus* (STEPH.)

Temperature	R. H., %	No. of eggs not hatched	No. eggs hatched	No. of eggs hatched after exposure of:					No. larvae that died	No. adults emerged	Average developmental period, days
				1 Day	2 Days	3 Days	4 Days	5 Days			
80° F.	10	32	3	0	2	1	0	0	3	0	—
	25	6	29	7	6	8	7	1	29	0	—
	50	5	30	4	7	6	10	3	10	20	37.7
	65	7	28	3	8	6	7	4	1	27	35.9
	75	2	33	6	4	9	11	5	2	31	31.1
	90	1	34	8	2	5	13	5	2	32	35.2
90° F.	10	23	12	6	6	0	0	0	12	0	—
	25	22	13	1	8	3	1	0	13	0	—
	50	12	23	2	11	9	1	0	10	13	26.4
	65	8	27	7	8	12	0	0	0	27	23.1
	75	3	32	7	6	12	5	2	3	29	20 or less
	90	3	32	4	16	9	3	0	4	29	19.7

It may be seen from Table IV that no larvae survived at 10 and 25% relative humidities at the temperatures in question. When examining the hatching records it should be kept in mind that the eggs were laid under conditions of 75% relative humidity and a temperature of 80° F., and that their ages at the time of placing in the vials were not known. This means that eggs hatching in one day under the new conditions must have undergone most of their embryonic development before being exposed to these conditions. At 80° F. and 10% relative humidity only three eggs hatched; no eggs hatching after three days' exposure to the desiccating influence of 10% relative humidity. At 90° F. and 10% relative humidity 12 eggs hatched, but no eggs hatched after two days' exposure to this humidity.

Larval mortality is 100% at relative humidities of 10 and 25%, all the larvae dying in the first instar. At 50% relative humidity the mortality is 80% with most deaths occurring in the first instar. Humidities from 65 to 90% are favorable for survival and development of the larvae and pupae, as the mortality rate throughout this whole range was no greater than what might be considered the natural mortality rate.

From the standpoint of the total numbers of adults obtained from the eggs kept at humidities of 75 to 90%, the difference between the number emerged at 80° F. and 90° F. is so small as to be insignificant. But, on the other hand, if speed of development is considered, the same humidities at a temperature of 90° F. are much nearer to optimum conditions than at a temperature of 80° F.

The results also show that relative humidity affects the speed of larval development, the period of development decreasing with an increase in the relative humidity. To study this acceleration of development due to the increase in relative humidity, another experiment was set up in which daily observations were made so as to determine the exact length of the different stadia. Three-tiered trays were used to hold the individual rearing cages in the constant humidity chambers. Each tray was taken from the chamber for a brief period each day in order to make the observations. Thus each set of rearing cages was exposed to room temperature and humidity for about 20 min. each day. One egg was added to each rearing cage and the larvae were fed on thin cross sections of wheat made through the germ region so as to include most of the germ. These sections were placed in the cages in the different humidity chambers eight days before the eggs were added, so that their moisture content would reach equilibrium with the air in the chambers.

Humidity chambers providing 25, 50, 65, 90, and 100% relative humidities were used in the experiment and all were kept at a constant temperature of 90° F. Figs. 28 to 32, inclusive, graphically depict part of the life history of *Laemophloeus ferrugineus* at the last five humidities mentioned above. In the 25% relative humidity chamber all the larvae died in the first instar. The first four horizontal sections of each graph represent the duration of the corresponding stadia, while the last horizontal portion of each graph shows the length of the pupal period. "X" indicates the emergence of the adult. The vertical lines represent time in days. The number to the left of each graph is the rearing cage number.

Data from these investigations are summarized in Table V which gives the arithmetic means for the lengths of the four larval stadia, and the pupal period in days. A study of these data shows that the acceleration of development due to an increase in humidity took place during the feeding stages of the larvae, there being no speeding up of the rate of development during the pupal stage at any of the different humidities. The fact that the acceleration when the humidity is increased is relatively less in the fourth stadium than in the preceding stadia can be explained by the fact that the larva spends the last two or three days of the fourth stadium in the nonfeeding prepupal stage.

TABLE V

EFFECT OF RELATIVE HUMIDITY ON THE RATE OF DEVELOPMENT OF *Laemophloeus ferrugineus* AT 90° F.

Developmental stages	Length of stadia in days at R.H. of:				
	50%	65%	75%	90%	100%
First instar	6.25	5.15	4.05	3.55	3.72
Second instar	5.83	4.25	2.75	2.89	2.50
Third instar	6.25	4.70	3.00	2.89	2.61
Fourth instar	9.50	7.90	7.45	7 49	6.83
Pupa	4.58	4.60	4.45	4.17	4.67
From hatching to adult	32.41	26.60	21.70	20.84	20.33

Most of the increase in the rate of development took place between relative humidities of 50 and 75%, there being no significant acceleration at humidities above 75%.

It is evident from the results of these humidity studies that infestations of *Laemophloeus ferrugineus* can develop in grain stored under relatively dry atmospheric conditions.

EFFECT OF TEMPERATURE ON THE INCUBATION PERIOD OF *Laemophloeus ferrugineus* EGG

Small squares of cardboard bearing several fine grooves made with a sharp knife were placed in a rearing cage that contained several adults. The following day the cardboards were removed to another container and examined daily to observe the time of hatching of the eggs that had been deposited in the grooves. The larvae present were removed at each observation. This method did not give the exact incubation period, as some of the eggs when first removed may have been almost 24 hr. old, while some may have been only a few minutes, but it did determine the approximate period.

This procedure was followed at temperatures of 80°, 90°, and 100° F.

Of a total of 37 eggs kept at 80° F., 22 hatched in 4 days, 14 hatched in 5 days, and one did not hatch; of 83 eggs at 90° F., 60 hatched in 3 days, 20 hatched in 4 days, and 3 did not hatch; while at 100° F. out of a total of 32 eggs, 11 hatched in 2 days, 19 hatched in 3 days, and 2 failed to hatch. These results show that for each rise of 10 degrees in temperature the incubation period was shortened by about one day. At a relative humidity of 75%, eggs at 100° F. hatched in 2 to 3 days, at 90° F. in 3 to 4 days, and at 80° F. in 4 to 5 days.

EFFECT OF TEMPERATURE ON THE LENGTH OF THE LARVAL AND PUPAL STAGES OF *Laemophloeus ferrugineus* AT A CONSTANT RELATIVE HUMIDITY OF 75%

Five different constant humidity chambers were prepared so that each provided a relative humidity of 75%. Each chamber was equipped with a three-tiered tray on which were placed 20 individual rearing cages. A thick

Graphs showing the effect of different humidities upon the developmental rate of larvae and pupae of Laemophloeus ferrugineus (Steph.). (The horizontal bars at successive elevations show the duration in days of the first, second, third, and fourth stadia, and the pupal stage, respectively.) R.H. = Relative humidity.

FIG. 28. *Developmental graphs for Laemophloeus ferrugineus at a relative humidity of 50%.*

FIG. 29. *Developmental graphs for Laemophloeus ferrugineus at a relative humidity of 65%.*

FIG. 30. *Developmental graphs for Laemophloeus ferrugineus at a relative humidity of 75%.*

FIG. 31. *Developmental graphs for Laemophloeus ferrugineus at a relative humidity of 90%.*

FIG. 32. *Developmental graphs for Laemophloeus ferrugineus at a relative humidity of 100%.*

cross section through the germ region of a wheat kernel was used as food in each of the rearing cages. One egg was placed into each cage and daily inspections were made with a binocular microscope until all the adults had

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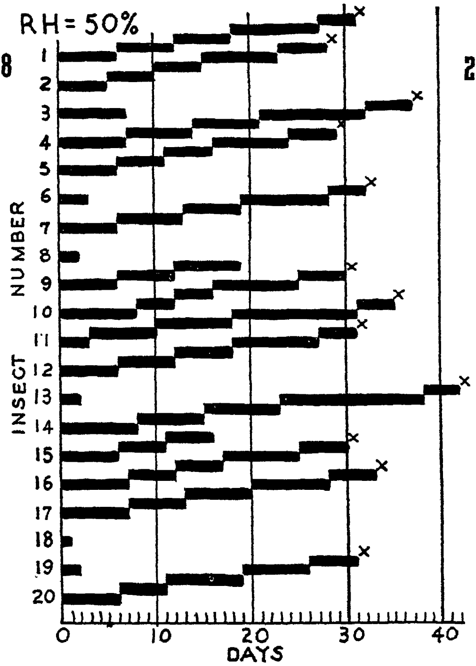

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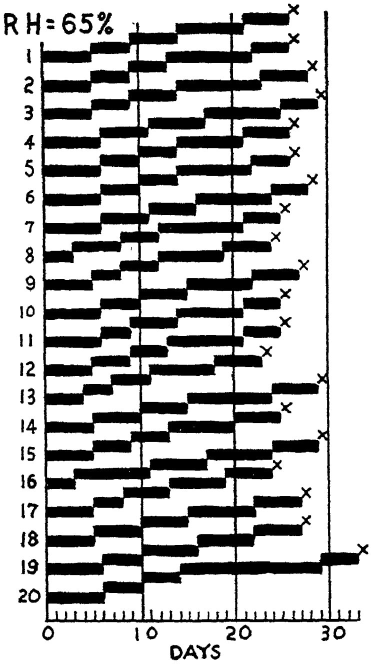

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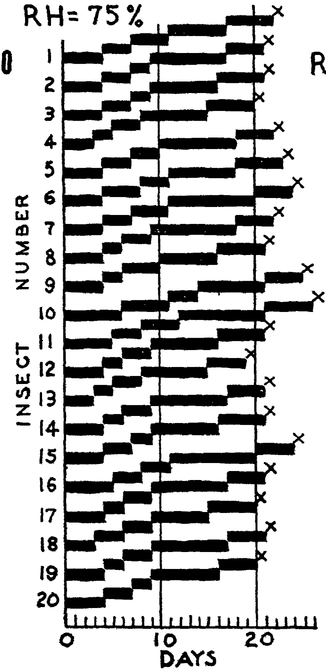

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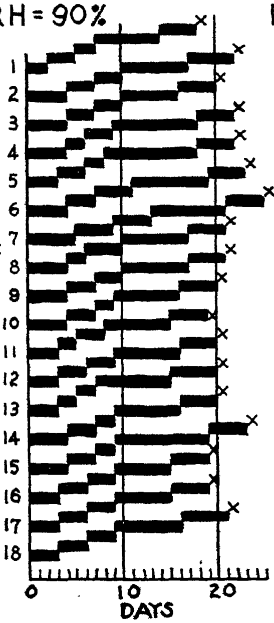

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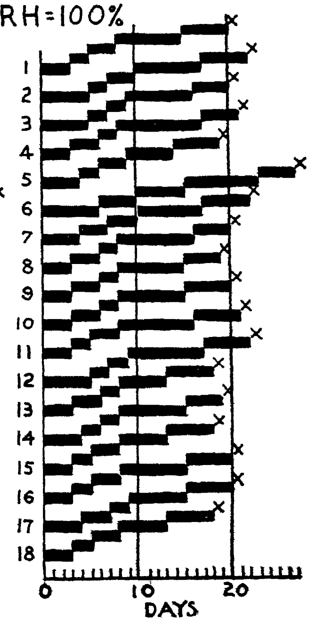


emerged. The discovery and removal of the cast-off exuviae from the container was the evidence relied upon in tabulating the time of molting. Each of the humidity chambers was kept in incubators at different temperatures so that records of the rate of development at temperatures 70°, 80°, 90°, 100°, and 110° F. were obtained. Figs. 33 to 36, inclusive, graphically depict part of the life history of *Laemophloeus ferrugineus* at the first four temperatures mentioned above. At 110° F. the eggs either failed to hatch or the larvae died immediately after eclosion. The first four horizontal sections of each graph represent the duration of the four larval stadia, while the last horizontal section of each graph shows the length of the pupal period. "X" indicates the emergence of the adult. The vertical lines represent time in days, and the number to the left of each graph is the rearing cage number.

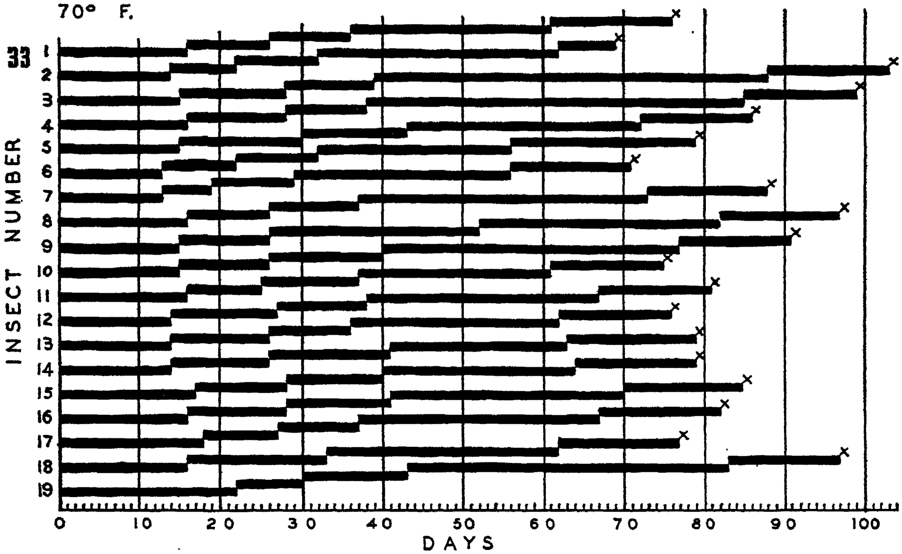
TABLE VI

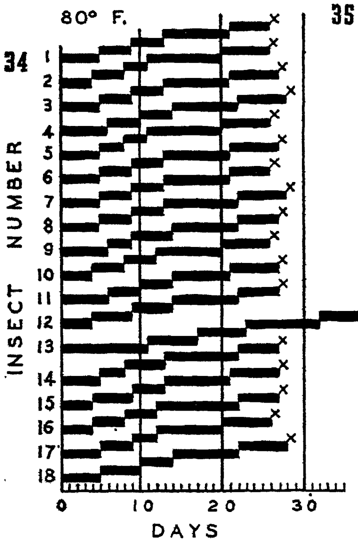
EFFECT OF TEMPERATURE ON THE RATE OF DEVELOPMENT OF *Laemophloeus ferrugineus* AT 75% RELATIVE HUMIDITY

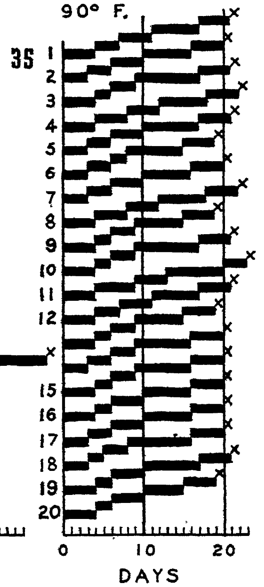
Developmental stages	Length of period in days			
	70° F.	80° F.	90° F.	100° F.
First instar	15.5	5.2	3.7	3.5
Second instar	10.6	4.4	2.1	2.8
Third instar	12.5	4.4	3.6	2.9
Fourth instar	30.9	8.3	6.7	8.6
Pupa	14.7	5.7	3.9	3.4
Developmental period from hatching of egg to adult	64.2	27.4	20.5	21.0

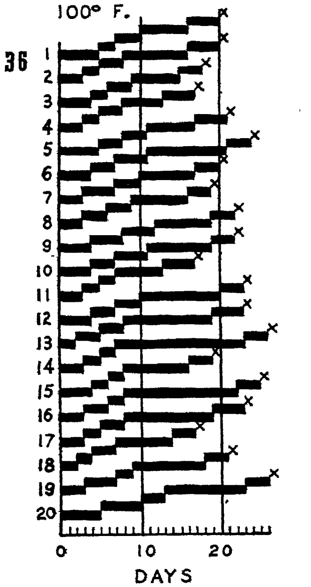
Data from these investigations are summarized in Table VI, which gives the arithmetic means for the lengths of the four larval stadia, and the pupal period in days. A study of these data shows that an acceleration in the rate of development occurred for each increase in temperature. An increase in temperature from 70° to 80° shortened the time from hatching of the egg to the emergence of the imago by 57 days, while an increase from 80° to 90° F. shortened this developmental period by seven days. An increase from 90° to 100° F. did not shorten the average length of this developmental period. The minimum developmental periods were 69, 26, 19, and 17 days at 70°, 80°, 90°, and 100° F., respectively, while the maximum periods for the same temperatures were 103, 38, 23, and 26 days, respectively. On the average it took more than four times as long for the rusty grain beetle to develop through the larval and pupal stages at 70° F. than it did at 90° F. Most of this great difference in rate of development occurred between 70° and 80° F.

The fact that development is not as uniform at 100° F. as at 90° F. may indicate that the optimum temperature has been passed when 100° F. is reached. This would mean that for the rusty grain beetle the optimum temperature for speed of development at 75% relative humidity is somewhere near 90° F.









Graphs showing the effect of temperature upon the larval and pupal development rate at a constant relative humidity of 75%. (The horizontal bars at successive elevations show the duration in days of the first, second, third, and fourth stadia, and the pupal stage, respectively.)

FIG. 33. *Development graphs for Laemophloeus ferrugineus at a temperature of 70° F.*

FIG. 34. *Development graphs for Laemophloeus ferrugineus at a temperature of 80° F.*

FIG. 35. *Development graphs for Laemophloeus ferrugineus at a temperature of 90° F.*

FIG. 36. *Development graphs for Laemophloeus ferrugineus at a temperature of 100° F.*

An interesting difference in the length of the various stadia is revealed in Figs. 33 to 36, inclusive, especially when the duration of the fourth larval instar stage at the four different temperatures is compared with the periods of the other instars at the same temperatures. The retarded speed of development of the fourth instar at 100° F., after extremely rapid development in the first three instars (see numbers 13, 14, 16, and 17; Fig. 36) suggests that at this high temperature the molting processes are so strongly stimulated that molting occurs ahead of other ontogenetic developments, and that the last molt is delayed in order that these other changes be allowed to catch up. It appears that in the process the total time required is actually as great or greater than that of those individuals that developed more slowly in the earlier instars. A possible explanation of this retarded development of the fourth instar larvae reared at 100° F. is contained in a report by Nelson and Palmer (8). They found that larvae of the flour beetle, *Tribolium confusum* Duval, must eat sufficient food to bring their phosphorus content up to a certain level before pupation would occur. It might be possible, therefore, that the very rapidly developing larvae of *Laemophloeus ferrugineus* did not assimilate during the first three stadia and the first two or three days of the fourth stadium enough phosphorus to permit pupation, and therefore longer feeding periods by the fourth instars were necessary to make up this deficiency.

It has already been mentioned that first instar larvae were not able to survive at a temperature of 110° F. and relative humidity of 75%. Since *Laemophloeus ferrugineus* has been recovered from heating grain at temperatures of at least 110° F. it was decided to determine what effect this temperature would have on the adults and older larvae. One hundred adult beetles, kept at 110° F. in an incubator in which a large evaporating dish filled with water was kept just below the electric fan at the top of the incubator, lived a normal existence for about one month until the evaporating dish was allowed to become empty. A few days later all the beetles were dead. Third and fourth instar larvae kept in the same incubator at the same temperature and high humidity were observed to remain alive for several days.

These observations suggest that adults of *Laemophloeus ferrugineus* at high relative humidities can tolerate higher temperatures than the first larval instars, and that all stages can live at high temperatures when the humidity is high.

Longevity of *Laemophloeus ferrugineus* Adults

On May 1, 1945, a single fourth instar larva was placed in each one of 100 small-sized rearing cages. The larvae were provided with cracked wheat as food, and kept at 90° F. and 65 to 75% relative humidity. On May 12, the cages were examined and an adult beetle was found in each. The 100 beetles were obtained in this manner so as to be sure that all the insects used were not more than a day or two old at the start of the experiment. This would not have been the case if beetles had been chosen at random from any of the stock cultures.

The cages were inspected once each month and a record kept of the dead beetles found. Table VII lists the results of these inspections and gives the total percentage dead at the end of each month.

TABLE VII

LONGEVITY OF *Laemophloeus ferrugineus* ADULTS FED ON CRACKED WHEAT AND MAINTAINED AT 90° F. AND 65 TO 75% RELATIVE HUMIDITY

Inspection date	Number of dead beetles	Percentage of total insects dead
May 12, 1945	0	0
June 12, "	0	0
July 12, "	2	2
Aug. 12, "	3	5
Sept. 12, "	4	9
Oct. 12, "	5	14
Nov. 12, "	13	27
Dec. 12, "	13	40
Jan. 12, 1946	37	77
Feb. 12, "	8	85
Mar. 12, "	11	96
Apr. 12, "	1	97

It will be seen from this table that at the end of the 11th month 97% of the beetles were dead, while at the end of the seventh month only 40% were dead. Thirty-seven per cent of the insects died in the eighth month of their adult life. We may conclude therefore, that at 90° F. and 65 to 75% relative humidity, and with cracked wheat as food, the adults of *Laemophloeus ferrugineus* live, on the average, from six to nine months, and a few may live as long as one year. This life span is somewhat longer than might be expected at a temperature as high as 90° F. and it almost certainly would be even longer at lower temperatures, where metabolic activities would be correspondingly reduced.

The longevity of adult beetles deprived of all food was also investigated. A single beetle was placed in each of 56 individual cages. Each cage had been thoroughly cleaned prior to the introduction of the imago. Table VIII gives the results of daily inspections of each cage to discover when its occupant died. The table shows that although one starved adult beetle lived as long as 17 days, more than one-half of the beetles were dead at the end of eight days. Seventy-one per cent of the beetles died between the 7th and 11th days inclusive.

The Suitability of Whole Wheat as Larval Food for *Laemophloeus ferrugineus* (Steph.)

A quantity of wheat, examined a kernel at a time to make certain that no damaged grains were included, was prepared. One-half of these undamaged kernels was soaked in water for five minutes and then removed and allowed to dry by exposure to the air in the laboratory. Later five kernels of the water-soaked wheat were put into each of 20 rearing cages. Into each of another 20 cages were placed five kernels from the unmoistened half of the undamaged wheat. All the cages were then put into an incubator running at 80° F. and 75% relative humidity, and left for a few days, in order that the moisture content of the grain might reach equilibrium with that of the air. Then one egg was placed in each cage. In the few instances in which the egg did not hatch, a new egg was added. The cages were inspected daily until all the eggs had hatched; then they were left undisturbed for 15 days when the kernels were examined for insect damage.

TABLE VIII

LONGEVITY OF STARVED *Laemophloeus ferrugineus* ADULTS AT 90° F. AND 65 TO 75% RELATIVE HUMIDITY

Inspection date	Number of dead beetles	Percentage of total insects dead
Feb. 8 (Expt. started)	0	0
" 9	0	0
" 10	0	0
" 11	2	3.6
" 12	1	5.4
" 13	4	12.5
Feb. 14	4	19.6
" 15	7	32.1
" 16	13	55.4
" 17	10	73.2
" 18	5	82.1
" 19	5	91.1
Feb. 20	2	94.6
" 21	1	96.4
" 22	0	96.4
" 23	1	98.2
" 24	0	98.2
" 25	1	100.0

This examination showed that all of the larvae except one had developed in a burrow in the germ region of the wheat kernel. The larval burrows were the result of the excavations brought about as the larvae fed on the wheat embryos. One larva had developed in the endosperm region in close proximity to the germ, a little of the germ being also consumed. There was no significant difference in susceptibility to insect attack between the water-soaked wheat and the untreated wheat.

The high rate of survival in the sound whole wheat does not agree with many reports in the literature that state that *Laemophloeus ferrugineus* is a secondary pest able to attack only grain that is already damaged by primary pests, such as the weevils and grain borers, or some other agent. The question of how the larvae actually gain access to the germ region then arose. Previously it had been noticed, while making dissections of wheat kernels from cultures of *Laemophloeus ferrugineus* being studied in the course of other investigations, that a certain proportion of wheat remained undamaged regardless of the beetle population or the duration of the infestations. Since five kernels were used in each of the rearing cages in the above experiment, each larva had to penetrate only one of the five to successfully complete its development. Thus there might have been kernels in each cage that could have withstood attack by the newly hatched larva. With this possibility in mind various samples of wheat were examined microscopically and it was discovered that very small breaks were discernible on many of the kernels, especially in the germ region.

In almost 50% of the "undamaged kernels" examined small breaks were found in the seed coats covering the germ region. Through these lesions the root cap of the radicle could often be seen. The Henry wheat used in this examination was obtained from the Wisconsin Experiment Station and had received more careful handling during harvesting and threshing than most farm grains receive. Furthermore, this grain had been stored under ideal conditions and had not been subjected to rough handling. Therefore, this sample could not be designated as damaged grain in the ordinary sense, although it had almost 50% of the kernels showing microscopic breaks in the bran layers. In order to determine whether *Laemophloeus ferrugineus* larvae could successfully attack intact kernels showing no lesions under microscopic examination, 278 whole undamaged kernels of Henry wheat were selected and examined carefully under the medium power of a binocular microscope. The embryo end of the wheat was examined very carefully. Any kernels showing the slightest evidence of a break in the bran layers were separated from those showing none. Twenty rearing cages were prepared and five kernels of wheat with broken bran layers were added to each cage. Twenty cages containing five kernels of wheat with no breaks in their bran layers were also prepared. Three eggs of the rusty grain beetle were placed in each cage. The cages were then put into the incubator and kept at 90° F. and 75% relative humidity. Nineteen days later the cages were removed and the kernels examined for insect damage. The results are recorded in Table IX. They show conclusively that the first instar larva of *Laemophloeus ferrugineus* is unable to attack whole wheat that has no breaks in the bran layers. The lone kernel damaged out of a total of 100 probably had a slight break in the germ covering that was missed during the microscopic examination. At least it cannot be assumed from this single exception that the larva had eaten its way through the bran layers. This table also shows that whole "undamaged" wheat that has small breaks in the layers over the germ region is easily attacked by first instar larvae. A total of 3 larvae, 33 pupae, and 1 adult was found in the cages containing the wheat with breaks in the bran layers.

TABLE IX

COMPARISON OF *Laemophloeus ferrugineus* DAMAGE TO WHEAT KERNELS WITH BROKEN AND UNBROKEN BRAN LAYERS OVER THE GERM REGION

Kernels with breaks in bran layer			Kernels with no apparent break in bran layer		
Cage No.	No. of kernels showing insect damage	No. of kernels showing no insect damage	Cage No.	No. of kernels showing insect damage	No. of kernels showing no insect damage
1	2	3	21	0	5
2	2	3	22	0	5
3	2	3	23	0	5
4	3	2	24	0	5
5	2	3	25	0	5
6	1	4	26	0	5
7	2	3	27	0	5
8	2	3	28	0	5
9	3	2	29	0	5
10	2	3	30	0	5
11	2	3	31	0	5
12	2	3	32	0	5
13	1	4	33	0	5
14	3	2	34	0	5
15	1	4	35	0	5
16	2	3	36	0	5
17	2	3	37	0	5
18	2	3	38	0	5
19	1	4	39	1	4
20	1	4	40	0	5

Suitability of the Different Parts of Wheat as Larval Food

Four experiments running concurrently under the same conditions of temperature and humidity (80° F. and 75% relative humidity) were conducted, the only variable being the food. The four lots A, B, C, and D, consisting of 20 rearing cages each, were provided with white flour, bran, a cross section of wheat containing wheat germ, and a cross section of wheat without germ, respectively. The lengths of the larval and pupal periods were recorded, and the larval periods are given in Table X.

A summary of the data of Table X is shown in Table XI. A glance at this table shows that wheat germ is by far the best larval food, whether percentage survival or rate of development is considered. Bran ranks second, with a mortality rate of only 11%, but the length of the larval period is more than doubled. White flour is next with a high mortality rate and a still longer larval period. Wheat with the germ end removed, although it contains the bran portion as well as the endosperm, is almost totally unsuitable as larval food. This indicates that the major reason for the high mortality in the latter case is the larva's inability to obtain nourishment because of the hardness of this portion of the wheat kernel.

The length of the pupal stage was the same for all four foods, the period averaging six days.

TABLE X

A COMPARISON OF THE DIFFERENT PARTS OF WHEAT AS LARVAL FOOD

Group A (white flour)		Group B (bran)		Group C (wheat germ)		Group D (wheat without germ)	
Rearing cage no.	Length of larval stage in days	Rearing cage no.	Length of larval stage in days	Rearing cage no.	Length of larval stage in days	Rearing cage no.	Length of larval stage in days
1	Egg didn't hatch	21	Died, 1st instar	41	22	61	38
2	66	22	65	42	20	62	34
3	Died, 1st instar	23	33	43	Killed	63	Died, 1st instar
4	Egg didn't hatch	24	Egg didn't hatch	44	21	64	“ “ “
5	Died, 1st instar	25	38	45	20	65	“ “ “
6	61	26	42	46	21	66	“ “ “
7	Died, 1st instar	27	36	47	22	67	“ “ “
8	“ 2nd “	28	60	48	20	68	“ “ “
9	“ “ “	29	51	49	21	69	“ “ “
10	Died, 1st instar	30	67	50	21	70	“ “ “
11	51	31	60	51	22	71	Died, 1st instar
12	54	32	Egg didn't hatch	52	22	72	“ “ “
13	58	33	79	53	Died, 1st instar	73	“ “ “
14	Died, 1st instar	34	66	54	20	74	“ “ “
15	“ 2nd “	35	Died, 1st instar	55	21	75	“ “ “
16	Died, 3rd instar	36	46	56	22	76	“ “ “
17	53	37	48	57	32	77	“ “ “
18	Died, 1st instar	38	65	58	22	78	“ “ “
19	49	39	47	59	21	79	“ 2nd “
20	Died, 1st instar	40	60	60	22	80	“ “ “

TABLE XI

COMPARISON OF DIFFERENT PARTS OF WHEAT AS FOOD FOR *Laemophloeus ferrugineus* LARVAE

Food provided	Mortality, %	Average length of larval stage in days
White flour	61	56
Bran	11	50
Wheat without germ	90	36
Wheat with germ	5	21.8

Susceptibility of Grain Kernels and Oil Seeds to Damage by *Laemophloeus ferrugineus*

Ten kernels each of Henry wheat, Imperial rye, Wisconsin Number 38 barley, Vicland oats, soybeans, a single hybrid dent corn, Redson flax, sunflower, and Zenith rice, were placed into each of 10 large-size metal rearing cages. The 90 seeds per cage did not completely cover the floor of the cage and as a result each seed was more or less isolated.

Ten unsexed beetles were added to each cage and the cages sealed with Scotch tape, treated with Tree Tanglefoot to prevent contamination, and placed in an incubator running at 90° F. and a relative humidity of 60 to 70%. The kernels in six of the cages were examined and the damaged kernels and insects present counted, 37 days after the start of the experiment, and those in the remaining cages after 71 days. The results are given in Table XII.

TABLE XII

SUSCEPTIBILITY OF VARIOUS GRAINS AND SEEDS TO DAMAGE BY *Laemophloeus ferrugineus* AT 90° F. AND 60 TO 70% RELATIVE HUMIDITY

Rearing cage number	Incubation period in days	Number of kernels damaged								
		Imperial rye	Henry wheat	Dent corn	Zenith rice	Vicland oats	Wis. No. 38 barley	Sun-flower	Flax	Soy-beans
1	37	6	1	0	0	0	0	0	0	0
2	37	7	2	2	0	0	0	0	0	0
3	37	7	2	0	1	0	0	0	0	0
4	37	6	4	2	0	0	0	0	0	0
5	37	4	0	0	0	0	0	0	0	0
6	37	7	4	2	0	0	0	0	0	0
7	71	6	2	1	0	0	0	0	0	0
8	71	8	4	0	0	0	0	0	0	0
9	71	5	2	1	0	0	0	0	0	0
10	71	6	2	1	0	0	0	0	0	0
Totals		62	23	9	1	0	0	0	0	0

A glance at this table shows that rye is the most susceptible to attack by *Laemophloeus ferrugineus* of any of the grains in this experiment. Wheat, corn, and rice were decreasingly susceptible, in the order named. Barley, oats, sunflower, flax, and soybeans were not damaged at all under the conditions of this experiment.

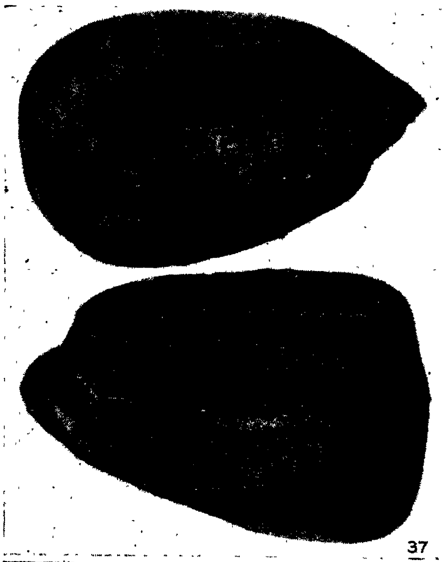
The high mortality rate among the adults, and the close correlation between the number of damaged seeds and the increase in the number of insects, as shown in Table XIII, indicate that most of the injury to the damaged kernels was caused by larvae rather than adults.

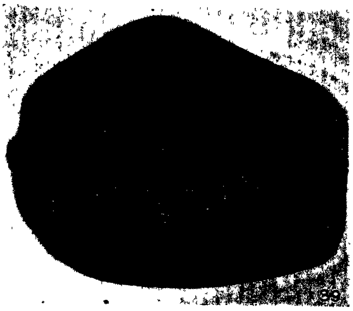
The type of damage caused by larvae feeding on kernels of corn, wheat, and rice are illustrated in Figs. 37, 39, 40, and 41.

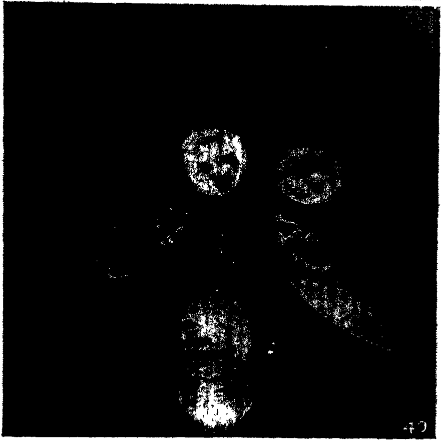
The Comparative Suitability of Damaged and Undamaged Grains and Oil Seeds for the Development of *Laemophloeus ferrugineus*

For this experiment the damaged grains and seeds were obtained by running them through a small hand corn-grinder. The grinder was adjusted so that the resulting meal was very coarse. The whole grains and seeds were considered as undamaged because they were obtained from grade one lots in first class condition.

PLATE I







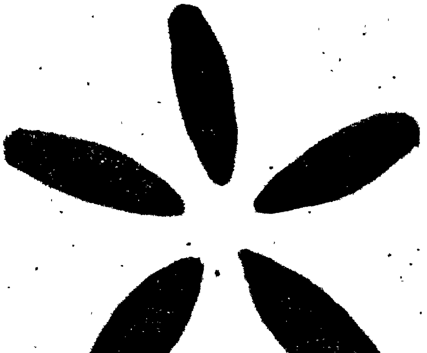


FIG. 37. *Typical damage by Laemophloeus ferrugineus larvae to the germ of Dent corn.*
FIG. 38. *Eggs of Laemophloeus ferrugineus.*
FIG. 39. *Adult of Laemophloeus ferrugineus emerging from a kernel of hybrid Dent corn.*
FIG. 40. *Wheat kernels degermed by Laemophloeus ferrugineus larvae.*
FIG. 41. *Rye kernels degermed by Laemophloeus ferrugineus larvae.*

TABLE XIII

POPULATION INCREASES OF *Laemophloeus ferrugineus* AND NUMBERS OF DAMAGED KERNELS IN A SERIES OF REARING CAGES

Rearing cage number	Dead adults	Live adults	Larvae	Pupae	Total population increase	Total damaged kernels
1	10	6	0	0	6	7
2	14	6	4	0	14	11
3	12	7	1	0	10	10
4	9	8	1	1	9	12
5	7	7	0	0	4	4
6	5	6	6	2	9	13

The large-size rearing cages were used. They were filled almost to the top and then heated to a temperature of 130° F. for 12 hours, after which they were allowed to cool. Twenty unsexed beetles were added to each cage, the lid sealed with Scotch tape, and the top treated with Tree Tanglefoot. The Scotch tape prevented book-lice, mites, or parasites from gaining entrance to the cages around the edges of the lids. The Tanglefoot kept any other pests from entering through the bolting-cloth-covered opening in the lid. The cages were kept for 47 days at a temperature of 90° F. and a relative humidity of 65 to 75%. Henry wheat, Zenith rice, Imperial rye, single hybrid Dent corn, Vicland oats, Redson flax, Wisconsin oats No. 219-12, soybeans, Blackhawk wheat, sunflower, and Wisconsin barbless barley were the foods studied. Two replicates of each food and each treatment were used.

A duplicate experiment was carried out exactly as above, with the exception that the foods were not heated prior to the introduction of the beetles, but instead were moistened with a few drops of water.

Total counts of the adults present in each cage were taken at the end of 47 days and the data obtained are given in Table XIV.

The total increases of adults on the different foods in all replicates are given in Table XV. However, it must be kept in mind while examining Tables XIV and XV that only adults were counted, and the fact that no increase is shown for some of the foods does not mean that *Laemophloeus ferrugineus* will not develop on them. For example, flax seed shows no increase in the number of adults, but many young larvae were observed in the damaged seed when it was being screened for adults. It should also be mentioned that in the case of the flax seed it was impossible, because of the similar color of the flax meal particles and the rusty grain beetles, to recover and count the dead beetles. The number of dead beetles listed for flax seed in Table XIV was derived by subtracting the number of live beetles recovered from the original number placed in each cage. The live beetle counts should be reasonably accurate, as they were secured by screening the foods, a small quantity at a time, into a

TABLE XIV

POPULATION INCREASES OF *Laemophloeus ferrugineus* IN VARIOUS WHOLE AND DAMAGED FOODS

Food	Condition of food	First run (heated)			Second run (moistened)		
		Beetles		Increase	Beetles		Increase
		Alive	Dead		Alive	Dead	
Henry wheat	Whole	236	15	231	260	17	257
	“	182	32	194	328	13	321
	Damaged	37	7	24	213	12	205
	“	63	6	49	241	9	230
Blackhawk wheat	Whole	52	14	46	216	13	209
	“	101	16	97	263	10	253
	Damaged	73	4	57	90	2	72
	“	48	11	39	117	1	98
Imperial rye	Whole	100	14	94	502	15	497
	“	190	15	185	248	11	239
	Damaged	45	2	29	247	8	235
	“	70	8	58	198	13	190
Vicland oats	Whole	1	19	0	6	14	0
	“	4	18	2	3	17	0
	Damaged	28	11	19	28	9	17
	“	45	4	29	171	6	157
Wisconsin oats 219-12	Whole	13	11	4	9	12	1
	“	0	20	0	21	6	7
	Damaged	35	4	19	36	2	18
	“	76	3	59	200	8	188
Wisconsin No. 38 barley	Whole	6	21	7	15	13	8
	“	18	14	12	4	16	0
	Damaged	81	10	71	81	14	75
	“	88	8	76	143	11	134
Zenith rice	Whole	51	26	57	82	10	72
	“	38	34	52	73	14	67
	Damaged	63	33	76	129	13	122
	“	170	22	172	39	6	25
Corn	Whole	12	18	10	17	9	6
	“	16	9	5	21	7	8
	Damaged	26	17	23	18	12	10
	“	37	14	31	37	14	31
Sunflower	Whole	7	13	0	10	15	5
	“	1	19	0	4	16	0
	Damaged	10	12	2	6	14	0
	“	16	10	6	7	13	0
Flax	Whole	0	20	0	4	16	0
	“	2	18	0	5	15	0
	Damaged	3	17	0	3	17	0
	“	1	19	0	2	18	0
Soybeans	Whole	0	20	0	0	20	0
	“	1	19	0	2	18	0
	Damaged	19	5	4	15	5	0
	“	20	7	7	14	8	2

large white evaporating dish. The live beetles were easily detected moving over the white surface of the dish and could be counted as they were sucked up into an aspirator.

TABLE XV

SUMMARY OF TOTAL INCREASES OF ADULT BEETLES FOR THE FOUR REPLICATES REPORTED IN TABLE XIV

Food	Insect increase	
	Whole food	Damaged food
Henry wheat	1003	508
Blackhawk wheat	605	266
Imperial rye	1015	512
Vicland oats	2	222
Oats 219-12	12	284
Wisconsin No. 38 barley	27	356
Zenith rice	248	395
Corn	29	95
Sunflower	5	8
Flax	0	0
Soybeans	0	14

It is quite certain that whole wheat or rye is more conducive to rapid increase in numbers of rusty grain beetles than are damaged kernels of the same grains. At first thought the reason for this seems obscure, but at least two factors may be responsible. It has been shown elsewhere in this report that larval development is much more rapid when the insect is feeding on germ than when it is feeding on endosperm. In the process of grinding, the germ portions become broken and dispersed so that the feeding larva, instead of finding enough germ at one location to complete its development, would be forced to move from place to place if only wheat germ were to be eaten. It is quite probable, therefore, that in the damaged grain a higher proportion of endosperm is eaten per larva than in the undamaged whole grain. This would result in a retardation in the rate of population increase. The second factor, namely the effect of cannibalism, may be of more importance than the suggestion just made. It has been observed on many occasions that the prepupae and pupae are very vulnerable to attack by larvae. In whole grain habitats the openings to the larval burrows usually are sealed with debris held together by silken strands just before the larvae enter the prepupal stage. This "debris plug" may be of importance in keeping female adults from ovipositing in the burrows, as well as in preventing migrating larvae from entering. It is not unreasonable to assume that cases of cannibalism might be much more numerous in damaged grain, where almost every prepupa and pupa is without the protection provided by a sealed larval burrow, than they are in undamaged grain.

Rye, Henry wheat, and Blackhawk wheat were the most satisfactory of the foods tested for the development of *Laemophloeus ferrugineus*. It has been shown elsewhere that rye is much more vulnerable to attack by larvae than wheat, and this experiment shows that the nutritive properties of rye are just as suitable for the insect as those of wheat. Therefore, in the case of wheat or rye, *Laemophloeus ferrugineus* cannot be considered as a secondary pest, depending on initial damage to the grain by other insects. However, in the case of oats, barley, corn, sunflower, flax, and soybeans it appears that the insect should be classed as a secondary pest. With the latter foods the populations increased more rapidly in the damaged grains, although in no case was the increase as great as that in wheat or rye. In fact, the whole kernels and seeds of this list were very resistant to insect attack and the very small increases shown in these whole foods may have been due to the presence of a few damaged kernels or seeds that are always present in any sample of grain or seeds. Where rice was used as food the results were about the same for the whole as for the damaged kernels, with the latter giving somewhat more rapid population build-ups.

Therefore we may conclude that under certain conditions *Laemophloeus ferrugineus* is not a secondary pest of rye or wheat, and possibly of rice, but that it may be considered a secondary pest of oats, barley, sunflower, soybeans, corn, and flax, when we define a secondary pest as one that must follow in the wake of some other insect pest if it is to be successful.

The Comparative Suitability of Weevil-infested and Undamaged Grain for the Establishment of Infestations of *Laemophloeus ferrugineus*

Ten rusty grain beetles were placed in each of four half-pint jars of wheat heavily infested with granary weevils, *Sitophilus granarius* (Linn.), and the same number of beetles were placed in each of four similar jars containing whole undamaged wheat. All eight jars were kept in an incubator at 90° F. and 65 to 75% relative humidity for 41 days, after which counts of adults were made. The results of these counts are given in Table XVI.

These results show that under the conditions of this experiment weevil-infested grain was no more favorable for the establishment of *Laemophloeus ferrugineus* than whole uninfested grain. However, it is interesting to note

TABLE XVI

NUMBER OF *Laemophloeus ferrugineus* ADULTS PER JAR

Jar No.	No. of *Laemophloeus ferrugineus* adults	No. of weevils	Condition of grain at start of experiment
1	215	430	Weevil-infested
2	86	278	" "
3	126	289	" "
4	128	311	" "
5	78	0	Undamaged
6	201	0	"
7	182	0	"
8	245	0	"

the apparent lack of competition between the two species, in spite of the large numbers of weevils present and the small size of the jars. This suggests that under low humidity conditions the presence of weevils may actually be an advantage to the rusty grain beetle, as has often been suggested in the literature. However, there would be no advantage in the case of wheat with a high moisture content.

Effect of Molds on Larval Development

Early experiments during the course of this work showed practically 100% mortality when larvae were supplied with cross sections of wheat that contained no wheat germ as the only source of food. Yet when supplied with bran or white flour the mortality rates were only 11% and 61%, respectively.

An experiment was set up to determine whether the food material contained in wheat that lacked the germ portion could be made available to larvae by the presence of molds. For this purpose three sets of 20 thick cross sections were prepared. Each section was examined under the binocular microscope to ensure that none of the wheat germ remained. The first set was left as an untreated control. The second set was soaked for 15 minutes in a solution of taka-diastase, made by adding 0.5 cc. of water to an amount of commercial taka-diastase that thinly covered the surface of a 10¢ coin. The third set was placed in an incubator at a high humidity until mold growth became luxuriant.

The sections from each set were then placed, one to each cage, into the individual rearing cages, which were then put in an incubator running at 80° F. and 75% relative humidity. After allowing one week for the grain moisture to reach equilibrium with the moisture of the air, an egg was placed in each cage and the period from the hatching of the egg to the emergence of the imago was recorded. The results are given in Table XVII. The 10% survival in the untreated control is in marked contrast with the 90% survival in the other two sets of cages.

The enzyme diastase acts on raw starch, dissolving it, and splitting it through the dextrin stage into maltose. However, not all the starch is converted entirely into maltose, a portion of it being left as dextrin. The maltose is usually hydrolyzed to glucose by the maltase present in the enzyme mixture. Thus in the case of the diastase-treated sections at least two changes resulted. First, a large proportion of the starch present in the sections was converted into forms that perhaps could be more readily assimilated by the larvae and, second, the sections were made considerably softer in texture. Which of these two changes was mainly responsible for the great decrease in mortality is not known.

However, this experiment showed that the survival of larvae fed on moldy sections was approximately the same as that of those fed on diastase-treated sections. It is well known that diastase is of wide occurrence among molds.

TABLE XVII

DEVELOPMENT OF *Laemophloeus ferrugineus* LARVAE ON THICK CROSS SECTIONS OF WHEAT CONTAINING NO GERM

Untreated sections		Taka-diastase sections		Moldy sections	
Cage No.	Period from hatching to adult	Cage No.	Period from hatching to adult, days	Cage No.	Period from hatching to adult, days
1	Died, 1st inst.	21	44	41	Died, 1st instar
2	“ “ “	22	46	42	52
3	“ “ “	23	39	43	32
4	“ “ “	24	44	44	32
5	44 days	25	39	45	38
6	Died, 1st inst.	26	38	46	34
7	“ “ “	27	39	47	30
8	“ “ “	28	44	48	34
9	40 days	29	36	49	32
10	Died, 2nd inst.	30	37	50	37
11	Died, 2nd inst.	31	40	51	Egg not hatched
12	“ 1st “	32	Egg not hatched	52	36
13	“ “ “	33	Died	53	35
14	“ “ “	34	“	54	49
15	“ “ “	35	35	55	35
16	“ “ “	36	42	56	45
17	“ “ “	37	43	57	44
18	“ “ “	38	43	58	36
19	“ “ “	39	43	59	42
20	“ “ “	40	39	60	Died, 1st instar

Summary

Treatment	Mortality rate, %	Period average (hatching to adult), days
Untreated control	90	42.0
Taka-diastase treated	10	40.6
Moldy	10	37.8

Whether or not the similarity of the survival rates obtained was due to the formation of diastase by the molds present was not established, but certainly the possibility of this being the case is a reasonable one.

This experiment definitely proves that the growth of molds upon a substratum of wheat endosperm converts the latter into more suitable larval food. From this it may be concluded that the presence of molds in stored wheat greatly increases the total amount of food suitable for the developing larvae by making the starch portion of the wheat more readily available as larval food.

Every larva that survived in both the moldy and diastase-treated wheat burrowed into the endosperm where it hollowed out a tiny cell in which it developed. Although both sides of the cross sections exposed large surface of the endosperm, many of the adults were held prisoners in their burrows, the openings being too small for the adults to escape.

It is of interest to note, while discussing the effect of molds upon larval development, that in order to obtain eggs in sufficient numbers for the various experiments carried out in the course of these investigations, it was necessary to keep the adults in wheat with a moisture content sufficiently high to encourage mold growth. Whether the high humidity alone, or the presence of molds as well, was a contributing factor causing this increased oviposition was not ascertained.

Larvae, adults, and eggs have frequently been found in abundance in cultures of wheat with such a high moisture content that the grain had been converted into a dark colored spongy mass by the action of the microorganisms present.

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STUDIES OF WATERFOWL IN BRITISH COLUMBIA

GREEN-WINGED TEAL[1]

By J. A. Munro[2]

Abstract

The migrations of the Pacific Coast population of *Anas carolinensis* parallel in time and space those of *Anas acuta*. Both use the same migration routes along the coast and along the main north and south river systems of the interior. So also both nest more commonly in Alaska than elsewhere. The peak of the spring migration usually is reached in late March or early April; the peak of the autumn migration usually is in late October and early November. A relatively large number normally winter on the Coastal Plain. Very few winter in the interior. Study of banding data reveals that (1) autumn transients through the Coastal Plain follow the coast route south to the mouth of the Columbia River and beyond through Oregon and California, rarely passing east of the High Sierras, (2) few individuals among the population following interior routes reach the coast, the tendency being to swing eastward to the Great Basin. The two main flyways through British Columbia, one on the coast the other through the interior, differ in at least one important respect, viz., along the interior route are many more or less isolated areas of suitable nesting grounds whereas on the coast there are none. A small nesting population, fluctuating annually in numbers, is widely distributed through the interior, the center of abundance being the Cariboo Parklands—the term abundance being used in a relative sense. Actually the population is small and dispersed. In the year 1938 a total of only 17 broods was counted on a study area of 60 sq. mi. containing a high average of highly productive waterfowl territory. Smaller counts were made here in each of the years following 1938. Egg-laying begins in May; there is some loss of early clutches through crow predation compensated for by later, and usually more successful, nesting. It is not unusual for females to be incubating a second clutch of eggs in early July. Nest sites are in dry places nearly always adjacent to a small pond or marsh in the grasslands. The earliest and latest dates for records of downy young are June 20 and Aug. 10. The average number of young in 48 broods counted in July, and 17 broods counted in August, was the same viz. 6.2. This high survival rate may be attributed in part to the spirited defense of young commonly practised by the female parent. Adult males in small number may associate on a common loafing place during the egg-laying period, and for a week or so after incubation has started. Later they retreat to marsh habitat and begin the yearly molt. Full eclipse of the body plumage usually is attained prior to renewal of the flight feathers. The molt from eclipse to nuptial dress is not completed until late October. The first adult plumage of young males is not fully attained until January. The sex ratio appears to maintain a not unfavorable biological balance—a total of 4264 banded green-winged teal was composed of 2225 males and 2039 females. The food range of the species is not extensive. Seeds, chiefly those of aquatic plants, were present in the stomachs of all 69 adults examined, and in many constituted the bulk of food eaten. Insects are second, miscellaneous animals third, in importance. The green-winged teal is a valuable game species; none of its habits is detrimental to agricultural or other human interests.

Introduction

The green-winged teal, *Anas carolinensis* Gmelin, is one of the more common pond ducks in British Columbia, its numerical status probably equal to that of the pintail, *Anas acuta* Linnaeus. It winters on the Coastal Plain of British Columbia and Washington, south through Oregon, California, and the

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Contribution from Dominion Wildlife Service, Department of Mines and Resources, Ottawa, Canada.

[2] *Dominion Wildlife Officer for British Columbia.*

southwestern States to Mexico. In more northern latitudes of British Columbia winter populations are small, and in southern interior waters only a few individuals remain through winter.

The southern limit of the nesting range is central California, where it is scarce (5) and southcentral Oregon, from where no recent records are

TABLE I

NUMBER OF GREEN-WINGED TEAL BANDED IN BRITISH COLUMBIA AND TOTAL RECOVERIES

Banding periods	Number banded				Total recoveries	Recovery, %
	♂	♀	Not sexed	Totals		
Station 1						
1931, Nov. 3 – Nov. 15	29	30		59	7	11.86
Dec. 8	9	4		13	1	7.69
1932, Oct. 16 – Oct. 30	70	106		176	20	11.36
Nov. 2 – Nov. 18	32	36		68	18	26.47
Dec. 4	2			2		
1933, Mar. 29 – Apr. 18	14	12		26	3	11.54
Oct. 4 – Dec. 12	240	184		424	70	16.51
1934, Jan. 28 – Feb. 2	67	75		142	3	2.11
Mar. 3 – Mar. 18	3	3		6	1	16.66
Oct. 17 – Dec. 3	55	62	128	245	36	14.69
1935, Mar. 10 – Apr. 5	131	110		241	24	9.95
Oct. 22 – Oct. 28			87	87	7	8.04
Nov. 11			15	15		
Nov. 27 – Dec. 13	3	2	83	88	4	4.54
1936, Jan. 7 – Jan. 25	7	7		14		
Mar. 13 – Apr. 6	86	68		154	13	8.44
Oct. 23 – Oct. 29	10	10	38	58	3	5.17
Nov. 7 – Dec. 3			289	289	14	4.84
Dec. 10 – Dec. 28	186	150		336	21	6.25
1937, Mar. 19	2	2		4		
Nov. 2 – Dec. 23			789	789	66	8.36
1938, Dec. 12 – Dec. 24			2	2		
1939, Feb. 2 – Feb. 22	3	2		5	1	20.00
Dec. 5 – Dec. 23			14	14	1	7.14
Station 2						
1933, Feb. 10 – Apr. 24	296	325		621	57	9.17
1934, Feb. 11 – Apr. 30	257	138		395	26	6.58
1935, Feb. 10 – Apr. 26	132	78		210	18	8.57
Station 3						
1932, Oct. 3 – Feb. 19, 1933			1201	1201	150	12.49
1933, Sept. 4 – Dec. 17			788	788	56	7.10
1934, Oct. 21 – Dec. 30	364	450		814	68	8.35
1935, Jan. 6 – Jan. 20	229	143		372	25	6.72
Station 4						
1931, Nov.			52	52	7	13.46
Station 5						
1932, Aug. 14 – Oct. 6			77	77	14	18.18
1933, Aug. 30 – Oct. 20			14	14	2	14.28
TOTALS	2227	1997	3577	7801	736	

reported (4). The northerly limit is northwestern Alaska, and it is from that territory that a large portion of the winter population on the Pacific Coast is derived.

Information relating to the distribution and migrations on the Pacific Coast Region, largely determined by the study of banding data, and observations of life history, behavior, and food habits in British Columbia are presented in this paper.

Five banding stations in British Columbia, operating at various times between 1931 and 1940, were successful to the extent of capturing and banding 7801 green-winged teal. Since then, 736 bands have been recovered, or examined on recaptured birds; 456 in the first 12 months after banding, the remainder as follows: second year—173; third year—54; fourth year—24; fifth year—13; sixth year—8; seventh year—4; eighth year—3; ninth year—1 (See Tables I to VII).

The geographical positions of the banding stations, and definitions of the terms used in discussing banding data, are presented in an earlier paper of this series (8).

TABLE II

DISTRIBUTION OF TOTAL GREEN-WINGED TEAL RECOVERIES

Locality where recovered	Number	Locality where recovered	Number
Alberta	4	Nebraska	1
Alaska	35	Nevada	4
British Columbia	315	Oklahoma	1
California	41	Oregon	49
Idaho	2	Utah	3
Montana	3	Washington	276
New Mexico	1	Yukon Territory	1
		Total	736

Distribution and Seasonal Movements

COAST REGION

The 7658 green-winged teal banded at Coast Stations 1, 2, 3, from 1931 to 1939, inclusive, resulted in a total of 713 recoveries as at Dec. 31, 1945. Of these 664 represent bands recovered from birds shot or otherwise killed, while 49 are records of birds retrapped at Coast Stations in subsequent banding seasons. Some of the last eventually were shot and again reported. Green-winged teal are on the Coastal Plain in numbers from late September until late March and the sum of banding operations over nine years covers this entire period. Thus the data from these operations provide a sampling of two seasonal movements, viz. (1) the autumn migration, September to November, inclusive, involving some birds that would winter on the Coastal Plain and others that would proceed farther south, and (2) the spring migration, February to April, inclusive, involving winter visitants to the Coastal Plain

TABLE III

RECOVERIES OF GREEN-WINGED TEAL, AUTUMN AND WINTER, ON COASTAL PLAIN, STATIONS 1, 3

Locality where recovered	Current recoveries	Later years	Locality where recovered	Current recoveries	Later years
Banded at Station 1					
British Columbia—			Washington Counties		
Station 1		4	Whatcom	7	4
Sumas–Chilliwack Region	23	8	Skagit	24	26
			Snohomish	5	3
Station 3	1		Island	2	4
Mouth Fraser River	13	8	San Juan	1	
Intermediate points	14	7	King	3	9
Pitt River Region	7	1			
Vancouver Island		5			
Banded at Station 3					
Station 3	7	27	Whatcom	1	3
Mouth Fraser River	18	28	Skagit	4	40
Vancouver Island	4	20	Snohomish		12
Station 1		1	Island	1	7
Station 2	1	3	San Juan		1
Pitt River Region	5	1	King	1	5
Intermediate points	2	2			
Sumas–Chilliwack Region	2	1			
	97	116		49	114

TABLE IV

RECOVERIES OF GREEN-WINGED TEAL, AUTUMN AND WINTER, SOUTH, EAST, AND WEST OF COASTAL PLAIN, STATIONS 1, 3

Locality where recovered	Current recoveries	Later years	Locality where recovered	Current recoveries	Later years
Western Washington counties			Eastern Washington counties		
Clallam	3	8	Chelan		1
Jefferson		2	Yakima		5
Kitsap	1	1	Okanagan		2
Pierce		3			
Mason	1	1	Western Oregon counties		
Gray's Harbour		4			
Thurston	3	2	Clatsop	4	9
Lewis		1	Columbia		3
Cowlitz		1	Washington		2
Clarke		4	Multnomah		5
			Marion		1
Utah State		1	Linn	1	1
Montana		1	Lane	2	2
Nevada	1	1	Coos	2	4
California	11	19			
			Totals	29	84

TABLE V

RECOVERIES OF GREEN-WINGED TEAL ON COASTAL PLAIN, AUTUMN AND WINTER FROM SPRING BANDING, STATIONS 1, 2, 3

Locality where recovered	First year	Later years	Locality where recovered	First year	Later years
Banded at Station 1					
British Columbia			Washington counties		
Station 1	3	2	Whatcom		3
Sumas–Chilliwack Region	2		Skagit	5	6
Pitt River Region	1		Snohomish		2
Mouth Fraser River	2	1	King	1	1
Vancouver Island	3	1			
Banded at Station 2					
Pitt River Region	6	5	Whatcom		1
Station 3	1		Skagit	12	8
Mouth Fraser River	7	4	Snohomish	2	1
Intermediate points		1	Island	2	3
Vancouver Island	1	4			
Banded at Station 3					
Station 3	15		Skagit	2	1
Mouth Fraser River		1	Snohomish		1
Intermediate points		2			
Vancouver Island	1	1			
Totals	42	22		24	27

TABLE VI

RECOVERIES OF GREEN-WINGED TEAL, SOUTH, EAST, AND WEST OF COASTAL PLAIN, FROM SPRING BANDING, STATIONS 1, 2, 3

Locality where recovered	First year	Later years	Locality where recovered	First year	Later years
Western Washington counties			Eastern Washington counties		
Clallam	1	7	Yakima		1
Jefferson		1			
Pierce		1	Western Oregon counties		
Gray's Harbour	1	1	Clatsop	3	2
Clarke	1	2	Columbia	1	1
Idaho State		1	Multnomah	1	2
Utah		1	Linn	1	
California	4	4			
			Totals	13	24

that were about to migrate, and others, from wintering places farther south, that had begun the journey to northern nesting grounds. As the periods of autumn and winter banding included the times in which it was lawful to hunt ducks, current recoveries were numerous during that season, but during the period of spring banding it was unlawful to hunt ducks so no current recoveries resulted.

TABLE VII

RECOVERIES OF GREEN-WINGED TEAL NORTH OF COASTAL PLAIN, STATIONS 1, 2, 3

Locality where recovered	First year	Later years
British Columbia		
West coast Vancouver Island	4	6
Mainland Coast	2	4
Goose Island	1	
Calvert Island		1
Graham Island		1
Southern Interior	3	
Central Interior	5	
Northern Interior	2	4
Yukon Territory	1	
Alaska	17	17
Alberta	3	1
Totals	38	34

In view of the large percentage of current recoveries resulting from autumn and winter banding, and the absence of current recoveries from spring banding, it has seemed advisable to tabulate the data in the following categories:

Table III.	Recoveries on Coastal Plain from autumn and winter banding, Stations 1, 3; current 146, later years, 230.
Table IV.	Recoveries, autumn and winter, south, east, and west of Coastal Plain, Stations 1, 3; current 29, later years 84.
Table V.	Recoveries on Coastal Plain from spring banding, Stations 1, 2, 3; first year 66, later years 49.
Table VI.	Recoveries, south, east and west of Coastal Plain from spring banding, Stations 1, 2, 3; first year 13, later years 24.
Table VII.	Recoveries north of Coastal Plain, Stations 1, 2, 3; first year 38, later years 34.

Autumn Migration

An early flight of no great volume takes place in September; the larger movements are through October and November. D. A. Munro counted 52 on Burnaby Lake, Sept. 21, 1946, and considered them to be the first autumn migrants (MS.). Later counts made by him at that place are: Oct. 6, 1946—305; Oct. 4, 1948—168 (in litt.). J. Tener counted 79 on Lulu Island, Sept. 10, 1947 (in litt.). It is of value to note here that in 1948 the earliest date of arrival in force on the Suisun State Waterfowl Refuge, San Francisco Bay Region, Calif., is Sept. 18, when 1200 were counted. Many populations travelling either by the coast or by the interior would seem to continue south with few stops en route.

The Coastal Plain, flanked on two sides by high mountain ranges, lies athwart a main highway of migration between northern nesting grounds and southern wintering grounds. Here large concentrations of ducks take place in a relatively small area and here migrating waterfowl are subject to an intense hunting pressure that is perhaps without parallel in any other section of the Pacific Flyway. It is not surprising then that 491, or 69.84% of total recoveries from coast banding come from this small geographical area. The times in which the largest numbers of bands were recovered can be assumed to be the times of maximum migratory movement. In three consecutive years this maximum was attained in November, as is shown in the tabulation below.

Number of recoveries on the Coastal Plain in three consecutive years

	October	November	December	January
1933	34	57	37	6 (1934)
1934	13	48	30	19 (1935)
1935	15	54	19	10 (1936)
Totals	62	159	86	35

Winter

The winter distribution of the green-winged teal on the British Columbia portion of the Coastal Plain parallels that of the pintail (9). The two species have similar food habits and a similarly limited food range, with preference shown for aquatic insects and seeds of both terrestrial and aquatic plants. The green-winged teal has not been observed feeding on waste grain in stubble fields, nor on stooked grain, as the more adaptable mallard does and in so doing extends the limit of its winter range. Nevertheless the green-winged teal does eat grain readily under some circumstances, for example when the grain is softened by water, or when it is available within easy reach of water. The natural food items are available in abundance only when flood ponds, shallow streams, and lakes remain open, a condition that, on the interior parts of the Coastal Plain, may persist throughout the winter in some years and at irregular intervals in others. Thus the species' movements within the region, and the size of the winter population in any year, are controlled by weather conditions to the same extent as are movements of the pintail.

The green-winged teal winter population is larger than that of the pintail so that its movements more often come under observation. Thus a shift of population from the Chilliwack–Sumas region to the coast, because of freezing conditions in the interior section, is sometimes obvious. Thus in 1933 a mass movement to the coast took place in early December, and a large part of the population sought refuge on an artificial lake at Station 3, near the mouth of the Fraser River, where at that time ducks were being fed some 600 lb. of barley each day. On Dec. 13 it was estimated that between 10 and 12 thousand green-winged teal, together with smaller numbers of mallard and pintail, were on this property. Station 1, near Chilliwack, had been closed on Dec. 12 because of cold weather and a scarcity of ducks. A similar western movement is revealed in the banding data for 1934. During the period Oct. 21 to Nov. 21 in that year banding results at Station 1 and Station 3 were comparable, viz., 244 and 309 respectively. On Dec. 1, after an unsuccessful week due to freezing conditions, Station 1 was closed; Station 3 continued to operate with greatly increased catches, a total of 804 green-winged teal being taken from Dec. 4 to Jan. 20.

Recent estimates of some local winter populations in British Columbia are available as a result of the "January Waterfowl Inventory" in which local ornithologists and Provincial Game Wardens participated. The figures for 1947, 1948, and 1949 are set out below:

	1947 (Jan. 7–17)	1948 (Jan. 7–10)	1949 (Jan. 11–14)
Coastal Plain			
Chilliwack–Sumas Region	5	19	0
Burnaby Lake	80	0	0
Fraser River Mouth to Boundary Bay	4500	3600	3200
Pitt Meadows	—	400	0
S.E. Vancouver Island	1600	300	500
Burke Channel, Fitzhugh Sound	—	300	300
West Coast, Vancouver Island	—	1300	—
Graham Island	100	100	—

Whatever weather conditions may prevail, the population wintering on the Coastal Plain, in recent years at least, is smaller than the population wintering on the Central Plain of California, usually a frost-free area and consequently an important wintering ground. The dates for first arrivals and for peaks of population at the Sacramento National Wildlife Refuge are set out below (Vernon Ekedahl, Refuge Manager, in litt.):

Year	Arrival in autumn	Number	Peak of population	Number
1943	Aug. 20	25	Dec. 22	4700
1944	" 16	250	" 19	25,000
1945	" 17	50	" 29	5300
1946	" 25	25		
1947			Sept. 22	13,600
1948	" 21	600	Jan. 8	58,210

Monthly totals of green-winged teal recovered along the Columbia River in southwestern Washington and Oregon, in southern Oregon, and in California are: October—9; November—31; December—49. Thus the arrival of these ducks in force on southern wintering grounds parallels in time the movement of large numbers from interior parts of the Coastal Plain to the sea coast, which usually takes place in late November and in December. An example of this movement, correlated with a sudden drop in temperature, was noted earlier.

The following are examples of green-winged teal banded at Stations 1 and 2 and recovered in the current year south of the Coastal Plain (see also Fig. 1):

Date banded	Locality where recovered	Date recovered
Oct. 16, 1933	Sutter Co., Calif.	Dec. 30, 1933
" 16, "	Sutter Co., Calif.	" 30, "
" 16, "	Thornton, Calif.	" 17, "
" 25, "	Sauvies Island, Ore.	Nov. 29, "
" 27, 1934	Butte Co., Calif.	Dec. 8, 1934
" 27, 1936	Seaside, Ore.	Nov. 2, 1936
" 30, 1932	Humboldt Co., Calif.	Dec. 24, 1932
Nov. 3, 1933	Suisun Marshes, Calif.	Dec. 27, 1933
Nov. 4, 1937	Modoc Co., Calif.	Dec. 17, 1937
" 7, 1932	Marshfield, Ore.	" 8, 1932
" 11, 1933	Santa Rosa, Calif.	" 28, 1933
" 11, 1932	North Bend, Ore.	" 11, 1932
" 21, 1934	Eugene, Ore.	Nov. 25, 1934
" 26, 1933	Clear Lake, Calif.	Dec. 25, 1933

It has been stated elsewhere (8, 9) that a large percentage of the recoveries of mallard and pintail were made on the Coastal Plain during the first few weeks after banding. The same high percentage of local early returns is observed in connection with the banding of green-winged teal. Thus, of 111 current recoveries of birds banded at Station 1, 91 are from the Coastal Plain, 28 being taken the first week, 21 in the second week, 11 in the third week, and 31 in periods of from four to nine weeks after the date on which the duck was banded.

Of the 28 first-week recoveries, 14 are from localities within a 20 mi. radius of the Station, two are from the Pitt River Region, 35 mi. west, four from places adjacent to the mouth of the Fraser River, 50 mi. west, one from Whatcom County, Wash., 30 mi. southwest, and seven from Skagit County—the farthest flight southwest being to Padilla Bay, 50 mi. distant from the Station, where one green-winged teal was shot two days, another seven days, after banding.

The 22 recovered during the second week came from the following localities: seven from points within a 20 mi. radius of the Station, four from the Pitt River Region, four from points near the mouth of the Fraser River, one from Snohomish County, four from Skagit County, and two from King County. The longest flight was to a locality 15 mi. south of Seattle, Wash., a distance of 120 air-line miles.

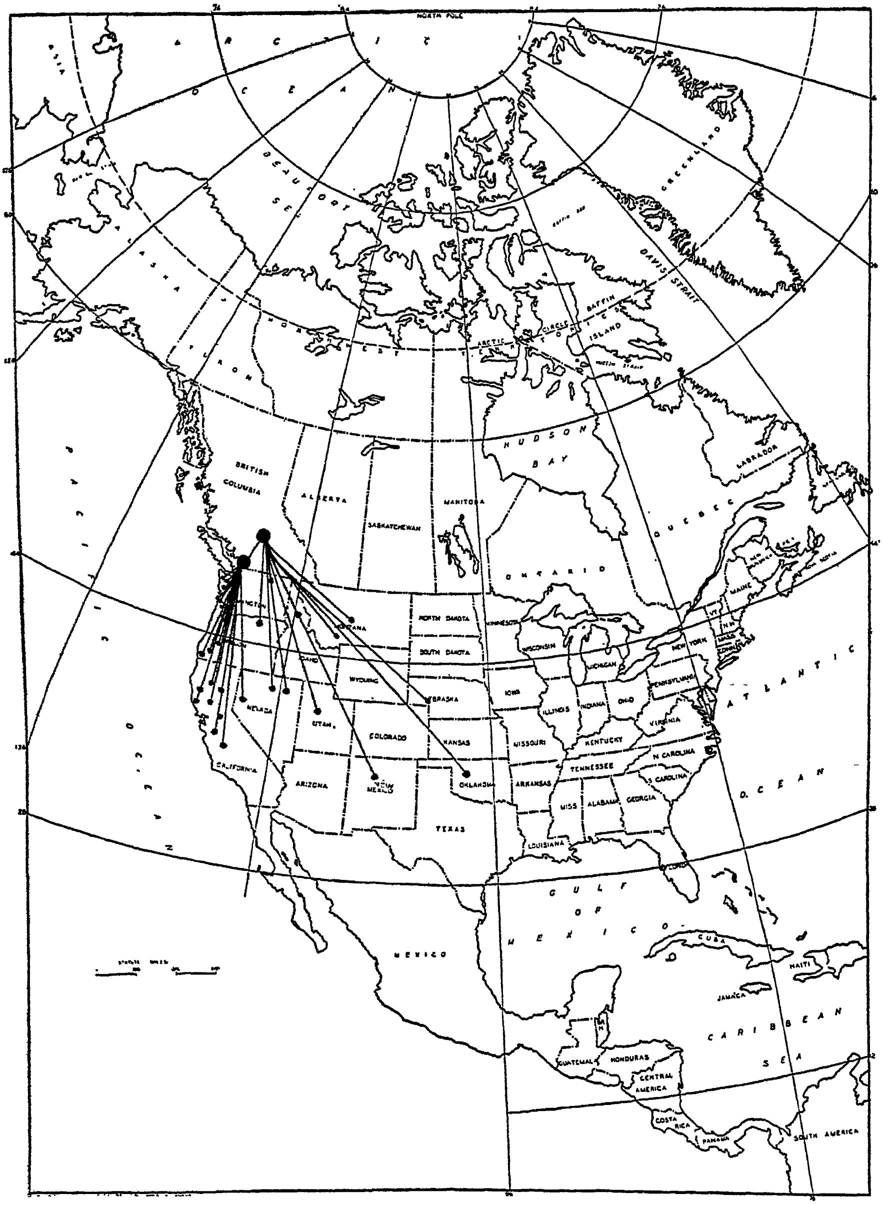

FIG. 1. *Current recoveries of transient green-winged teal banded at Stations 1, 3 (left), and Station 5 (right). Each dot represents one or more recoveries.*

Of the 11 bands recovered during the third week five are from localities within a 20 mi. radius, three from the Fraser River mouth, and one from each of the counties Whatcom, Skagit, and Snohomish.

The remaining 30 bands, recovered at later periods, come from the same general localities, all within an extreme distance of 120 mi.

To be considered here, also, are 246 recorded occurrences of green-winged teal recaptured at Station 1 during the current banding season in the years 1932–1937, inclusive. One hundred and thirty-seven were taken one to six days after banding, the remainder after elapsed periods approximately as follows: one week—43; two weeks—27; three weeks—19; four weeks—10; five weeks—8; six weeks—1; seven weeks—1. The total of 246 is a fraction of a larger number captured but not recorded.

The foregoing data give some idea of the extent to which winter populations move from place to place within the Coastal Plain. In addition the data designated as current trap-recoveries afford evidence as to the length of time certain couples or groups remain within the vicinity of the Station. For example: two banded Nov. 12, 1936, were recaptured Dec. 3, 1936; three banded Nov. 19, 1937, were recaptured Dec. 12, 1937, and two banded Nov. 6, 1937, were recaptured Dec. 10, 1937. It may be inferred that these particular groups wintered in the vicinity of the Station.

Four instances of a westerly flight over the Gulf of Georgia from Station 3 to Vancouver Island are recorded, the particulars being as follows:

Date banded	Locality where recovered	Date recovered
Jan. 23, 1933	Ladysmith	Jan. 1933
" 10, 1935	Victoria	Jan. 21, 1935
Jan. 13, 1935	Crofton	Jan. 18, 1935
Jan. 13, 1935	Clayquot	Jan. 30, 1935

As was done in a study of the pintail (9) the subsequent history of certain green-winged teal that have been recovered and that represent the total of returns from one day's banding at a single station has been assembled. Three samples of this grouped data follow.

Station 1

A total of 40 recoveries are reported from a group of 142 green-winged teal banded Nov. 4, 1939. In the same year 14 were recaptured and released at Station 1 on Nov. 6; four were recaptured on Nov. 8, and single birds were recaptured on each of the following dates: Nov. 13, Nov. 29, Dec. 4, and Dec. 11. One was taken again on Nov. 3 and Dec. 8, another on Nov. 6, Dec. 8, and Dec. 12. The following were shot: one at Lulu Island, Nov. 5; one at Matsqui, Nov. 22; one at Sumas (B.C.), Dec. 11; six in Skagit County, Wash., between Nov. 9 and Dec. 3, and one in Modoc County, Calif., on Dec. 17. In 1938 one was shot Nov. 13 at St. Helena, Columbia County, Ore.; another on Nov. 8 at Vancouver Lake, Wash.

Station 2

A total of eight recoveries are reported from a group of 137 banded on Mar. 10, 1933. In 1933 one was recaptured and released at Station 2 on Apr. 11; one was shot at Pitt River on Oct. 12, another at Camano Island, Wash. on Oct. 22, and two at Ladner on Nov. 11. In 1934 one was shot at Pitt River on Jan. 14, one at Valdez, Alaska, on Sept. 14, another at Sauvies Island, Ore., on Dec. 7.

Station 3

A total of 20 recoveries are reported from 242 green-winged teal, banded on Jan. 13, 1935. Four were shot later in the same month, two near the banding station, one at Clayquot Sound and another at Crofton, Vancouver Island. In the following May one was recovered about 200 mi. north of Hazelton, B.C. In the autumn and winter of 1935 six were shot on the Coastal Plain, one at the mouth of the Fraser River, one at Pitt Meadows, two near Olympia, Wash., on the same date, viz., Oct. 23, and two in Skagit County, Wash. In 1936 two were taken at Shawnigan Lake on Feb. 18, and one was shot at Chemainus on Nov. 23—both these places are on Vancouver Island.

A total of 26 recoveries have been reported from an unknown number banded at Station 3 on Feb. 5, 1933. One was shot at Kanatak, Alaska, the following April and in the autumn and winter of that year one was taken at Goose Island, one at Fort Simpson, B.C.; two in Skagit County, Wash.; one in Clallam County, Wash.; three in Clatsop County, Ore.; 11 were recaptured and released at Station 3 on the following dates, viz., Oct. 21—1; Nov. 1—1; Nov. 26—4; Dec. 3—3; Dec. 10—2; and one was shot near the Station on Nov. 12. In the autumn and winter of 1934 one was shot in Skagit County, one in Clark County, Wash.; one near New Westminster, B.C.; and one near Nanaimo, Vancouver Island. In 1935 one was shot in Snohomish County, Wash., Nov. 2, and one near Surrey, B.C., on Dec. 1.

Many green-winged teal that escape early destruction return to the same localities in succeeding years. Table III shows that 67 in a total of 230 subsequently returned to Stations 1 and 3 where they were banded, or to places within a 20-mi. radius of these Stations. When assembled in year groups the data reveals that 35 were recovered in the autumn and winter of the first year, that is to say after they had made one journey to and from their nesting grounds, and 32 were recovered in the second year. None were taken subsequently at or near these stations.

The date on which green-winged teal were banded in one year and the date on which they were recovered in the following year are, in some instances, in the same month, close enough to suggest an approximate time of arrival in each year, the difference in time perhaps being modified in some degree by differences in the prevailing weather conditions. While the first seasonal recorded appearance of an individual does not necessarily mean that the bird had just arrived, nevertheless there is a strong possibility of this being so. For the first few days after units of population reach the Coastal Plain, possibly because of a recent protracted flight and a degree of exhaustion resulting from it, ducks are commonly much less wary than at other times, so consequently are more eager to enter banding traps where food is available, and also much more likely to be shot.

The following records are of green-winged teal banded at Station 3 and recaptured there, or shot within a 20-mi. radius, in the following year.

Date banded	Date recovered	Date banded	Date recovered
Dec. 8, 1932	Dec. 3, 1933	Dec. 18, 1932	Dec. 3, 1933
" 11, "	Nov. 19, "	" 20, "	Nov. 19, "
" 15, "	Oct. 29, "	" 20, "	Dec. 3, "
" 15, "	Nov. 2, "	" 20, "	" 3, "
" 15, "	" 26, "	" 20, "	" 10, "
" 18, "	" 11, "	Nov. 5, 1933	Oct. 24, 1934
" 18, "	" 19, "	" 5, "	Nov. 14, "
" 18, "	" 26, "	" 11, "	Oct. 24, "

The above is acceptable evidence that units of populations return to the same localities on the Coastal Plain, a conclusion borne out by numerous instances of couples or groups that, banded together on the same day, were later recaptured together, some after a period of several weeks.

Number in groups	Date banded	Date recaptured
2	Mar. 12, 1933	Apr. 18, 1933
7	" 19, 1935	Mar. 25, 1935
2	" 19, 1935	Apr. 1, 1935
4	" 25, 1935	Apr. 1, 1935
2	Nov. 12, 1936	Dec. 3, 1936
3	" 19, 1936	Nov. 29, 1936
3	" 19, 1936	Dec. 10, 1936
14	" 4, 1937	Nov. 6, 1937
3	" 4, 1937	Dec. 13, 1937
2	" 6, 1937	Dec. 10, 1937

Population cohesion is illustrated with equal emphasis by other data in reference to couples, or groups, which return to the Station at which they were banded after an absence of one year. All the examples shown in the tabular material on p. 162 are of green-winged teal banded at Station 3.

The summaries indicate that the large flocks of green-winged teal visiting the Coastal Plain are composed of aggregations of small populations each of which exhibit definite cohesion. It can be anticipated that extensive banding operations along the flyways and on nesting grounds will add further evidence in support of the conclusion advanced here.

Spring Migration

A concentration of green-winged teal on the Coastal Plain prior to migration has been observed and is apparent in banding data. Thus at Station 2 in 1933 the daily total of birds captured increased from 13 on Mar. 9 to 137 on Mar. 10 and 47 on Mar. 11. The number dropped to 12 the following day, and thereafter the largest daily catch until Apr. 13, when operations ceased, was 29 on Apr. 9. Similarly at Station 1 in 1935 the largest number taken during a four-day period in spring, Mar. 19 to 22, was 114. Subsequently, until the Station was closed on Apr. 15, a total of only 71 was captured.

Number in groups	Date banded	Date recaptured
2	Nov. 27, 1932	Oct. 29, 1933 Dec. 3, "
3	Dec. 11, "	Oct. 21, " Nov. 19, " (2)
2	Dec. 15, "	Oct. 29, " Nov. 26, "
5	Dec. 18, "	Nov. 11, " " 19, " " 26, " Dec. 3, " Jan. 20, 1935
4	Dec. 20, "	Nov. 19, 1933 Dec. 3, " (2) " 10, "
4	Jan. 29, 1933	Nov. 11, 1933 " 26, " Dec. 3, " " 10, "
11	Feb. 5, "	Oct. 21, " Nov. 12, " " 26, " (4) Dec. 3, " (3) " 10, " (2)

Field observations indicate that, by the first week in April, the main migration has passed, leaving only a few small units and finally most of these also leave for northern nesting grounds. There is a lack of observational data concerning the spring migration along the northern coast, there being but two counts available, both from the Queen Charlotte Islands, viz., McClinton Creek, Apr. 15, 1935—30, and Tlell, May 5, 1935—20.

Summer

Summer records of green-winged teal on the Coastal Plain in British Columbia are not uncommon. For example, a small population frequents Burnaby Lake and, in 1946, the numbers were recorded throughout the summer, the largest counts being May 21—8, June 12—4, July 6—7, Aug. 19—7 (D. A. Munro, MS.). Another population is reported from Lulu Island where the following counts were made in 1947: May 14—6, June 2—1, June 14—3, June 17—4, July 4—11, Aug. 1—7, Aug. 25—16, Aug. 26—35, Aug. 27—22, some of the last probably being transients (John Tener, in litt.). The investigators were of the opinion, after close study of these two areas, that neither population included any breeding pairs. Green-winged teal are reported to have nested in the vicinity of Vancouver (3) and in the vicinity of Chilliwack (1), but in neither account is satisfactory evidence submitted. It seems clear then, that all, save a small residue probably composed of non-breeding birds, migrate beyond the limits of the Coastal Plain. Banded green-winged teal have been taken at many places on the coast and in the interior of Alaska, indeed a majority of the recoveries (89.5%) from north of the Coastal Plain come from there (see Table VII), and there is little doubt that Alaska is the principal nesting ground of the west coast population. The localities in that territory, and the number of bands recovered at each place are: Akiachak—1; Becharof Lake—1; Bristol Bay—1; Cook Inlet—2; Chulitna River—1; Dillingham—1; Goodnews—1; Iditarod—1; Kaltag—1; Kenatak—1; Katalla—1; Koyuk—1; Koyukuk—2; Kuskokwun River mouth —1; Kwigillingok—1; Mountain Village—1; Nunachuk Village—1; Perryville—1; Pilot Station—1; Sitka—2; St. Michael—1; Steelmute—1; Seward Peninsula—1; Tanana Crossing—1; Togiak River—1; Valdez—2; Yukon River—2; Yukon Delta—2.

INTERIOR REGION

Spring Migration

As compared with the spring migration from the Coastal Plain along the coast to Alaska the migration north through the Interior is relatively small and some of the elements composing it are members of populations that nest in southern latitudes. The two main flyways, one along the coast the other across the Interior Plateau, have this important difference, viz., over much of the interior route are suitable nesting grounds used by this species, whereas along the coast there are none.

Through the southern part of the Province there appear to be five streams of migration tributary to the main flyway across the Interior Plateau. Populations that have wintered on the Coastal Plain follow the Fraser–Thompson system and the Columbia–Okanagan system; the two flight lines come together in the grassland region adjacent to Kamloops.

There is evidence of a third route, one via Howe Sound, Squamish, Pemberton Meadows, Anderson, and Seton Lake, but about this no precise data are available.

The Rocky Mountain Trench and the Purcell Trench in eastern British Columbia are migration routes used by populations of green-winged teal that apparently are distinct from those wintering on the Coastal Plain. There have been two recoveries on the Kootenay Flats, in the Purcell Trench, of green-winged teal banded at Malheur Lake in eastern Oregon, viz., one banded Apr. 5, 1933, was taken at Creston, Sept. 20, 1936; another banded Mar. 26, 1936, was taken at Wynndel, Nov. 4, 1938. None of the 7801 green-winged teal banded in British Columbia have been taken in the eastern portion of the Province.

The first of the Eastern routes mentioned is known to follow the Columbia Valley, of which the southern portion contains many suitable resting and feeding places, at least as far north as Golden. It perhaps may be assumed that the route then follows the general course of the river north across the Columbia Forest Biotic Area, where no suitable feeding places exist, to the Big Bend, and from there, where the Columbia River turns south, in a northwesterly direction probably following the Canoe and Fraser Rivers to the ponds and marshes of the Cariboo Parklands and to the grassland and aspen country beyond. The second route follows down the Kootenay River where resting and feeding places on the Kootenay Flats temporarily detain the ducks, to Kootenay Lake, and north over the swampy valley of the Lardo. After leaving the favorable territory of the Kootenay River and its tributaries, this route, also, presumably bears to the northwest.

There is little information as to the numbers of green-winged teal that pass through the Cariboo Parklands in the early part of any spring migration. A flock of 247 counted at Little White Lake, Apr. 29, 1938, was considered to be the last of the spring flight for that year.

At Chezacut Lake, in the Chilcotin District, the earliest records are of 40 on Apr. 18, 1940, and 20 on Apr. 23, 1941. In the Lakes District of Vanderhoof, farther north in the Central Interior, the migration had passed through prior to May 1, 1945, except a few belated flocks, of which one of 10 and another of 17 were noted on May 6. The only spring banding returns from the Central Interior Region is that of a male banded at Station 1 on Apr. 11, 1933, and killed that spring on Lake Uncha, near Danskin, which is on Francois Lake.

Spring migration data in more northern parts of the Province refer to Tetana Lake where, in 1941, the first was seen on Apr. 8 (11) and to Atlin where the earliest spring records are reported to be Apr. 30, 1933, Apr. 20, 1934, and Apr. 22, 1935 (14).

The particulars of three banded green-winged teal recovered in northern British Columbia are:

Banding Station	Date banded	Locality where recovered	Date recovered
Station 1	Dec. 4, 1937	Buckley Lake, 20 mi. SE. Telegraph Creek	Spring, 1938
Station 2	Mar. 4, 1935	Rolla, 12 mi. N. Peace River	Apr. 23, 1935
Station 3	Dec. 20, 1935	Driftwood River, near Takla Landing	May 14, 1937

In summary, the spring migration of the green-winged teal through the Central Interior follows closely in time behind that of the pintail, with the maximum number in transit between Apr. 1 and Apr. 15.

Autumn Migration

The rapid flight of green-winged teal, and the uniform timing of movement of the individuals composing a flock, characteristic of the species at any time, seems more apparent, and a source of admiring comment, during the autumn migration. Very often these ducks travel in small flocks of 10 to 30 individuals and a number of such units, usually composed of the season's young, may combine to form a company of 100 or more. Such a transient flock may assemble on a muddy shore, and nervously alert, may rise suddenly, ascend to a height, fly in a wide circle, descend spirally with a sharp whistle of wings, wheel out over the water, then alight once more—all these movements being performed with the speed, the grace, and the precision of a flock of sandpipers. Green-winged teal associate freely with mallard and with pintail, occupy the same mud bars, tidal flats, and other loafing places, and feed under cover in the bulrush marshes that mallard frequent.

The first wave of the autumn transients usually appears in early August, often two weeks or a month earlier than the first transients appear on the Coastal Plain.

Observations and records of the autumn migration through the interior are summarized in the following paragraphs:

Atlin. The latest recorded observation of green-winged teal in autumn is Oct. 5, 1931 (14).

Kispiox Valley, on the Kispiox River at a point 23 mi. north of Hazelton. In 1921 the first autumn transients were observed Aug. 10 (13).

Vanderhoof. In the Lakes District in 1945 a flock of 60± was observed on a marshy slough, Aug. 31. In 1946 the first indications of a southern migration was a flock of 25 in flight over Sinkut Lake, Aug. 25. There are two banding returns from this locality, viz., one banded at Station 2, Mar. 23, 1934, was shot at Nulki Lake, Sept. 12, 1934; another banded at Station 1, Nov. 6, 1937, was shot on the Nechaco River, Oct. 29, 1938. Thus the three records give a possible migration period from Aug. 31 to Oct. 29.

Cariboo Parklands. From the 91 green-winged teal banded at Buffalo Lake, Station 5, in the autumn seasons of 1932 and 1933 (See Table I), 16 bands have been reported—one from Alaska; two from near the mouth of the Columbia River in Washington State; and 13, of which 12 are current recoveries all but one from southern interior localities. These data, being of exceptional interest in that they show a first-season wide dispersal over the Great Central Basin and eastward (see Fig. 1), are submitted in full, as shown in the tabular material at the bottom of p. 166.

Buffalo Lake, in the Cariboo Parklands, is on the main flight-line of the interior, north of where three tributary routes converge, thus the high percentage of banding returns from eastern localities is significant evidence of the small extent to which the Coastal Plain wintering population is represented on the interior flight-line (see Fig. 1). There is added significance in the fact that only 14 bands from green-winged teal banded at Coast Stations have been recovered in the interior of British Columbia. The details of those recovered in the Cariboo Parklands follow:

Station where banded	Date banded	Locality where recovered	Date recovered
Station 1	Nov. 13, 1932	Gang Ranch	Sept. 27, 1933
Station 1	" 3, 1933	North Bonaparte	Autumn, 1934
Station 2	" 26, 1933	Soda Lake, 108 Mile Ranch	Oct. 11, 1934

Okanagan Valley. The earliest evidence of an autumn migration in any year refers to a flock of 75 on Goose Lake, near Vernon, Aug. 10, 1919.

The adjacent Swan Lake is the most important resting and feeding place for green-winged teal in the Okanagan Valley and here the maximum autumn concentration usually takes place during the period Sept. 15 to Oct. 15; more rarely it is not attained until early November. The following enumerations are of maximum numbers recorded in each of nine years: Sept. 15, 1932—300±; Sept. 30, 1933—400±; Oct. 4, 1934—275±; Nov. 5, 1942—200±; Oct. 10, 1943—30±; Sept. 10, 1946—41; Oct. 6, 1948—120±.

Fifty-two green-winged teal were banded in Nov., 1931, at Vaseaux Lake, in the southern part of Okanagan Valley, and seven bands were recovered later. Two were local, current recoveries, and two local recoveries in Dec., 1932; one records the interesting information that a male banded on Nov. 7 was shot 28 mi. north of the banding Station on Dec. 3. One is a second year recovery from California, and another records that a male banded Nov. 7 was shot in Klamath County, Ore., on Dec. 20 of the same year.

Date banded	Locality where recovered	Date recovered
Aug. 25, 1932	Yakima Co., Wash.	Dec. 2, 1932
" 25, "	Albuquerque, N. Mex.	" 10, "
" 30, "	Hat Creek, Nev.	Nov. 19, "
Sept. 1, "	Pacific Co., Wash.	Jan. 1935
" 2, "	Holitna River, Alaska	May, 1934
" 2, "	Cache Co., Utah	Oct. 8, 1932
" 2, "	Whitefish, Mont.	" 2, "
" 4, "	Los Angeles, Calif., 40 mi. south	Nov. 1933
Sept. 6, 1932	Nelson, Calif.	Dec. 12, 1932
" 15, "	Kalispell, Mont.	Oct. 1, 1932
" 16, "	Hot Springs, Idaho	Jan. 16, 1932
" 16, "	Nye Co., Nev.	Oct. 1, 1932
" 17, "	Wahkiakum Co., Wash.	Oct. 1932
Oct. 7, "	North Powder, Ore.	Oct. 23, 1932
Sept. 19, 1933	Kingfisher, Okla.	" 22, 1933
" 19, "	Valley, Neb.	" 13, "

West Kootenay. At Sirdar Lake on the Kootenay Flats two autumn migrations were tallied as follows:

1947		1948	
Date	Number of birds	Date	Number of birds
Aug. 7	6	Aug. 19	23
“ 26	350±	“ 27	85
“ 29	250±	“ 29	270±
Sept. 8	600±	Sept. 5	80
“ 10	800±	“ 8	70
“ 11	350±		

East Kootenay. Dates for early arrival of transients at Golden in the Columbia Valley in 1948 are: Aug. 17—20; Sept. 6—48 (D. A. Munro, in litt.).

It will be valuable at this point to consider data in reference to migrations south of the International Boundary. The first wave of autumn migrants usually reaches the Tule Lake National Wildlife Refuge in northern California, and the adjacent Lower Klamath Lake National Wildlife Refuge in southern Oregon, sometime during the last two weeks of August, peak numbers normally being recorded in October. Later with the first prolonged cold spell comes an abrupt decline in numbers and relatively few remain through winter (Howard Sargeant, Refuge Manager, in litt.). Dates of arrival in force and dates of maximum numbers are tabulated below.

First arrival in force	Peak numbers attained
Tule Lake National Wildlife Refuge	
1945	Oct. 28—50,000
1946 Aug. 31—5000	“ 15—20,000
1947 “ 31—2000	“ 10—18,000
1948 Sept. 20—2500	“ 31—15,000
Lower Klamath Lake National Wildlife Refuge	
1945 Sept. 10—2500	Oct. 28—10,000
1946 Aug. 31—3000	“ 15—25,000
1947 “ 31—2500	“ 10—45,000
1948 “ 30—3500	“ 20—20,000

Summarizing the above section it can be said that in general the autumn migration through the interior takes place from early August to late October; a large migration in November was observed once only. Autumn and winter current recoveries of green-winged teal banded at Buffalo Lake suggest that the largest number using this flight-line are transients to and from southern interior localities, while a much smaller number is associated with the Coastal Plain.

Reproduction

In summer the green-winged teal is widely distributed through the interior of British Columbia from near the International Boundary to Atlin in the extreme northwest part of the Province. The populations are small, particularly so in the south from where but few nests or broods have been recorded. A female accompanied by eight young three-quarters grown was observed on the Kootenay Flats, Aug. 18, 1947, and three nests with eggs are reported from the east Kootenay, viz., two at Cranbrook Slough, examined by W. B. Johnstone, June 8, 1939 (MS.) and one found near Fairmont Hot Springs, June 14, 1948 (D. A. Munro, in litt.). It is reported to have nested near Edgewood, in the west Kootenay (6) and there are three records of broods from the Okanagan Landing region, viz., one of half-grown young, June 23, 1915, and two late broods of downy young, Aug. 10, 1919. No recent breeding records from the Okanagan Valley are reported.

The Cariboo Parklands in southcentral British Columbia attract a relatively large nesting population, but one that shows marked fluctuation in numbers from year to year. The following record of broods observed, while by no means indicating the totals of population in different summers, reflects in some measure the extent of this fluctuation.

1936	1937	1938	1939	1941	1942	1943	1944	1948
2	6	39	7	2	2	2	2	2

On a sample study area of 60 sq. mi. containing the most productive nesting grounds, the total counts were 45 adults and 17 broods in 1938, and 12 adults and two broods in 1948.

On the Endako River drainage small numbers have been found nesting, or with broods, in the vicinity of Vanderhoof, Francois Lake, and Ootsa Lake. A summer population is reported from the Driftwood Valley, approximately 175 mi. to the northwest of Vanderhoof (12) and, at Swan Lake and Charlie Lake in the Peace River Block, five broods of young were counted, June 8, 1938 (2). At Atlin, where the species is reported to be a "common summer resident", a brood of downy young was observed, July 13, 1934 (14).

During the mating season pairs of green-winged teal frequently have been flushed, or have been seen rising in display flight, from areas of open water hidden by dense marsh growth. It seems likely that courtship, other than display flight, is commonly practised in such concealed places; however that may be, no mated birds, nor groups of both sexes, have been observed in courtship display on beaches or on open water. Neither has any hostility among the males been noted. During the laying season it is not unusual to see several males idling on the surface of some secluded marsh pond, or standing together on its muddy shore, on a half-submerged log, or on some other slight prominence as they await the arrival of the females from the nests. The actions of the males, and their apparent lethargy, in the laying season is in marked contrast to the behavior of both blue-winged teal and cinnamon teal, which are aggressive among themselves and constantly court and display in full view of a human observer.

In the Lakes District, Vanderhoof, during May, 1945, pairs, and trios composed of two males and one female, were frequently seen in courtship-flight, and in the latter part of May and early June it was common to see mated pairs together, the latest date recorded for this association being July 1.

Nesting

The preferred nesting habitat of the green-winged teal is one of grassland, sedge meadow, or dry hillside containing aspen or brush thickets, or open woods adjacent to pond or slough. The number of eggs in a clutch varies from six to nine, with an average of eight. (Broods of 10, 11, and 13 have been recorded, but these may be the product of more than one family. Thus at Mirage Lake, Aug. 1, 1938, one female led a brood of 13, another a brood of seven, while a third was accompanied by only one young.) No nests have been found at a distance greater than 250 yd. from water and usually the distance is much less; all those examined were in dry situations. The following examples are typical.

Horse Lake, Cariboo, May 10, 1943. Habitat, narrow belt of willows, alders, and aspen near the shore of a marshy bay; site, on open ground close to a dwarf birch, *Betula glandulosa*, at the inner margin of the tree belt; nest, a depression in the ground 6 in. in diameter containing a few dead leaves of dwarf birch and alder. It contained four eggs on May 10, five eggs on May 12, and six eggs on May 13, the last time the nest was examined.

Slough near Westwick Lake, Cariboo, July 3, 1948. Habitat, edge of aspen woods 60 ft. from small forest pond in which small sedges, grasses, and *Sagittaria* hid the water; site, under cover of procumbent juniper, *Juniperus scopulorum*; nest of dry grass and down; nine eggs.

Springhouse Prairie, Cariboo, July 8, 1941. Habitat, dry sedge meadow beside a small pond; site, in sedges 100 ft. from water; nest, a slight depression lined with dry sedge stems and a large amount of down around the eggs, and around the circumference of the nest to a height of 3 in. above the surface of the ground; eight eggs.

Manson's Slough, near Vanderhoof, June 17, 1945. Habitat, dry sedge meadow adjoining slough; site, in sedges 150 yd. from water; nest of dry grass mixed with down and feathers; six eggs, four of which had been destroyed by crows.

Tatley Slough, near Fairmont Hot Springs, East Kootenay. Habitat, dry, wooded hillside above slough; site, 250 ft. from slough against a small Douglas fir trunk, of which one branch 2 in. above ground level provided shelter; nest of grass and small amount of down set in bowl approximately 6 in. deep and 7 in. in diameter; eight eggs subsequently eaten by a predator, probably a crow (D. A. Munro, in litt.).

Crow Predation

Instances of green-winged teal eggs eaten by crows have been mentioned. This predation would seem to occur more often in the early weeks of summer when nesting cover often is inadequate. In each of four raided nests the eggs had been fresh and the clutch incomplete; in one were two eggs, which, deeply buried in the down, remained intact. Clutches of eggs laid in June or early July, when cover vegetation has reached an advanced stage of growth, are much less vulnerable. Crow predation can be considered an adverse factor in green-winged teal reproduction in that a percentage of early-laid eggs are destroyed and that subsequent clutches, representing a second nesting, usually are smaller.

Behavior of Nesting Females

Before leaving the nest the female covers the eggs with down, if this has been added to the nest, otherwise with whatever material composes the nest lining. Thus when the nest at Horse Lake, described above, was first examined the eggs were covered by a few dry leaves, many of which lay in the nest cavity and on the ground beside it. Covered thus the eggs were effectually concealed and the nest completely merged with the surrounding litter. This particular teal was flushed three times from the nest at distances of 20 to 30 yd.; each time she uttered a thin, quacking note continuously as she flew. The nest, as has been noted, was situated at the inner edge of a tree-belt, which provided effective barrier between it and the water. The teal, upon leaving the nest, did not attempt to rise over the barrier but flew close to the ground for 50 yd. or so to where an opening led to the water, and through this opening she passed.

The eggs in the nest at Springhouse Prairie, noted earlier, were in an advanced stage of incubation. On July 8 and July 9 the setting teal did not flush until I had approached within 12 ft. of the nest.

Behavior of Female with Brood

Female green-winged teal defend their broods in a seemingly fearless manner, which the following examples illustrate, the first being the characteristic behavior when a brood is concealed in a lakeshore marsh and its hiding place is approached by canoe. At Watson Lake, Cariboo Parklands, June 30, a female rose from the marsh and flew out over the water, passing me within a few yards, then ascending slightly, turned about and made a short circle back across the marsh, presumably over the place where the young were hiding. She then flew directly towards me again and alighted within a few yards of the moving canoe there to swim ahead, sometimes in the ordinary manner, sometimes beating the water with extended wings. These actions were accompanied by a continuous, excited quacking. Meanwhile the brood completely eluded observation.

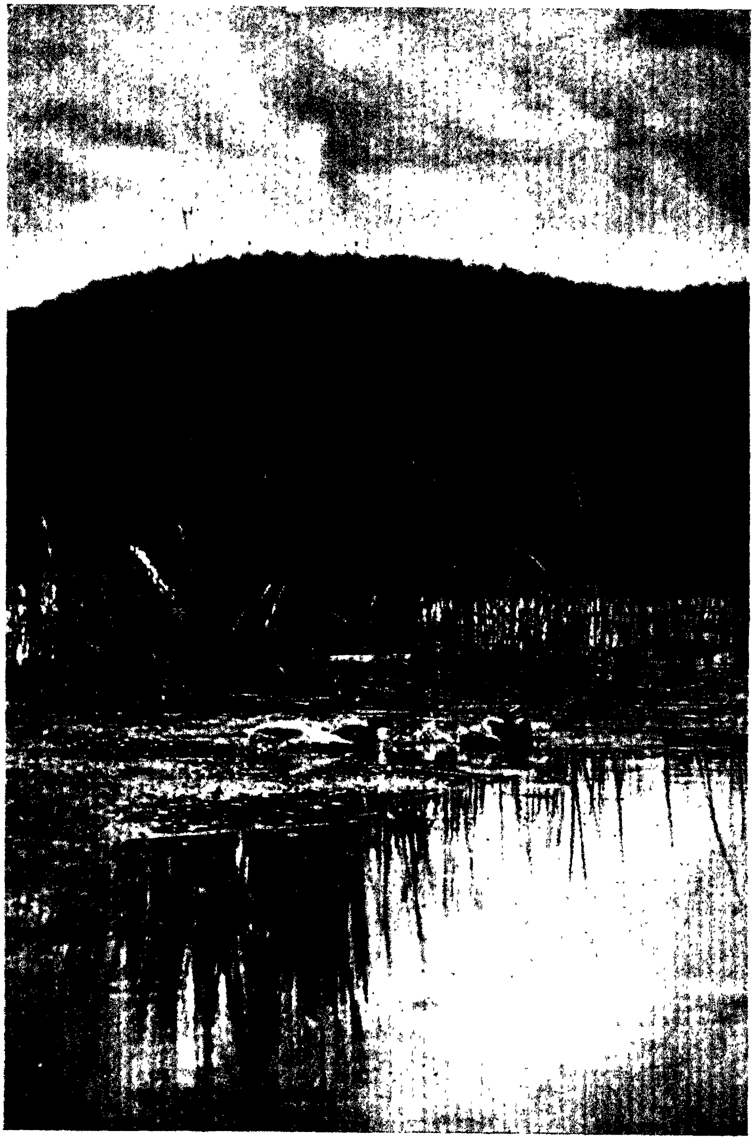

FIG. 2. *Two female green-winged teal defending their combined broods,*

PLATE II

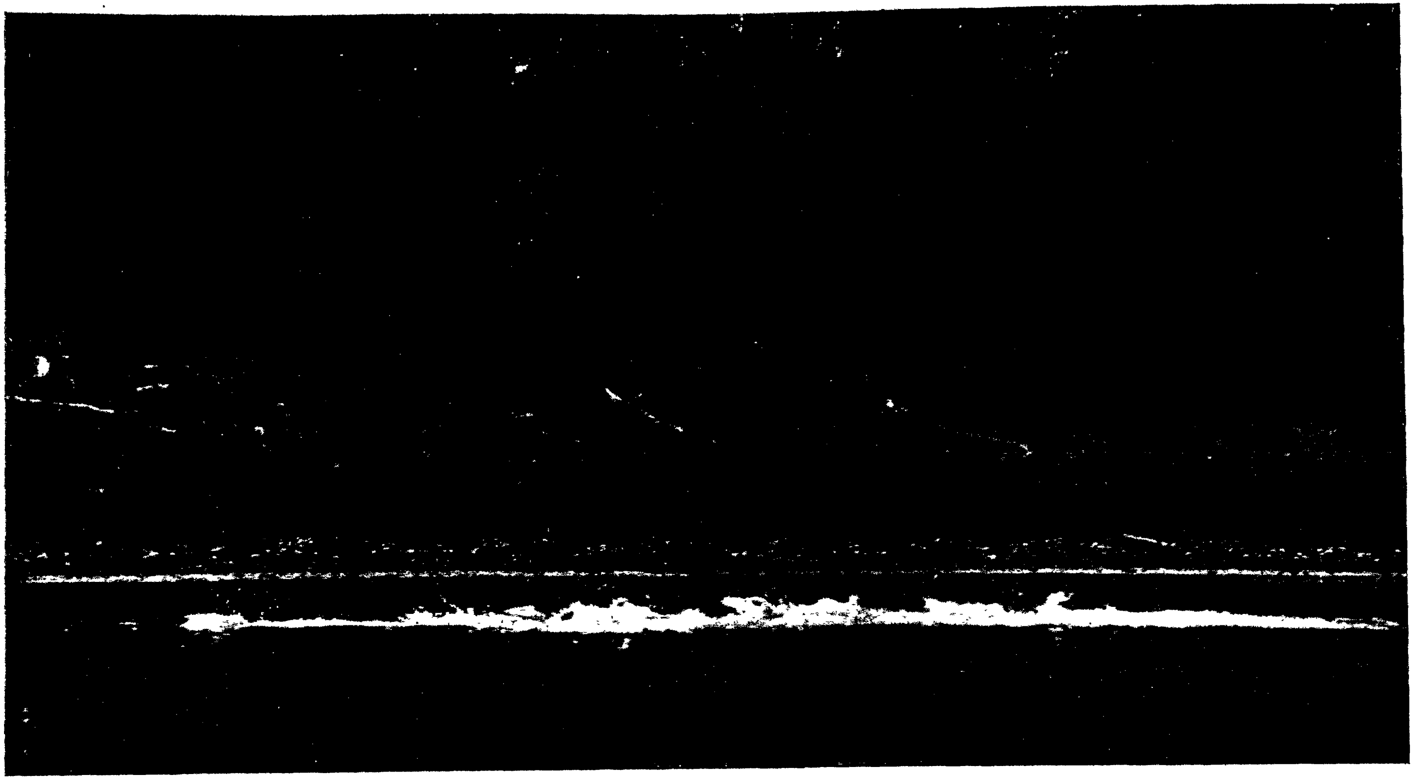

FIG. 3. *Brood of green-winged teal, about three-quarters grown and flightless, retreating to shore cover.*

At Westwick Lake, July 7, 1941, a female surged back and forth across a small area of shallow water on the margin of the lake while her brood of six young, in a compact flock, swam out toward the lake's center. This incident took place while I stood on shore. On the same day, at another smaller lake, a female flew out from a thick clump of rushes, and, alighting beside my canoe, made the usual demonstration. A few minutes later a brood of eight young, about two weeks old, rushed out from the same section of cover and dispersed over the surface of the lake. After a few minutes the brood reformed and swam along the marsh edge while the female swam between it and my canoe.

The behavior of a female with brood when approached by a man on foot is altered to suit this different situation; she then attempts, by fluttering along the ground with one or both wings dragging, to draw attention away from the brood.

Several females with broods may combine under the stress of excitement caused by human intrusion, and together defend their young. One such instance involving three females and a group of 19 young has been recorded elsewhere (10) (Fig. 2). Another example is that of three females accompanying a band of 14 half-grown young on Pete Kitchen Lake, Cariboo Parklands, July 18, 1938. A third, illustrated by Fig. 3, concerns two combined broods rushing to shore cover on Mirage Lake, while the females perform defense actions on the water.

The defense tactics of the female green-winged teal when directed towards a man cannot conceivably be of any survival value, for in most instances the presence of a brood in the marsh growth would not have been detected had the female remained with it. It is conceivable, however, that had they resulted from the presence of a different type of predator, for example a coyote hunting along the edge of the marsh, the tactics would have definite survival value.

The earliest and latest dates for the first records of downy young are June 20, 1940, and Aug. 10, 1919, respectively.

The average number of young in 48 broods counted in July and 17 broods counted in August was the same, viz., 6.2. The high average survival may be attributed in part to the vigorous defense reactions of the females.

Postbreeding Behavior, and the Eclipse Plumage, of Males

It is usual to find several adult males associated on a common loafing place even during the early part of the laying period, and such places they may continue to occupy for the first week or so of incubation. By this time some males have begun to molt, and the bright colors of the nuptial dress is approaching an eclipse. Soon all males have retreated to the seclusion and comparative safety of a marsh habitat so necessary to survival during the flightless period of molt that is to follow. Thus in the Lakes District, Vanderhoof region, in the summer of 1945, all males had disappeared from open water by the first week in July. One instance of an adult male in full eclipse in company with a female and seven half-grown young was observed at Lily Pad Lake, July 21, 1939.

In the Cariboo Parklands males begin to assume eclipse plumage in late June and early July. There is much individual variation in the time of molt. Thus, at 149 Mile Lake, July 19, 1939, two males still were in full nuptial plumage—so far as could be determined by careful scrutiny with 8× binoculars, while at Lily Pad Lake two days later two other males were observed to have attained full eclipse. On July 13, 1943, all of four adult males, flushed together from a round-stem bulrush marsh at 103 Mile Lake, and nine males flushed singly from the marshes at Watson Lake on July 23, were in full eclipse. None has been observed during the flightless period that, presumably, occurs with most individuals from late July to early August.

The molt of the contour feathers from eclipse to nuptial plumage is a slow process, with flank feathers the last to be renewed. A specimen from Swan Lake, Okanagan, Oct. 14, 1939, retains eclipse feathers among the greater wing coverts, and, in the finely vermiculated nuptial plumage on the flanks, eclipse feathers are conspicuous. The molt of young males is continued over a still longer period. A specimen from Swan Lake, Sept. 28, 1936, another from Okanagan Landing, Oct. 15, 1920, show no adult feathers. One from the last locality, Dec. 12, 1930, retains a few juvenile flank feathers, and juvenile feathers predominate on the back.

Sex Ratio

A total of 4264 green-winged teal, with sex recorded, was banded at Coast Stations; of these 2225, or 52% were males, and 2039, or 48% were females. On 119 of the 255 banding days the number of males exceeded the number of females; on 32 days the proportion of the sexes was equal, and on 104 days the number of females exceeded the number of males.

The greatest daily sexual disproportion is indicated by the following figures: males in excess of females 34/11, 36/23, 66/40, 30/18, 167/75, 37/13, 24/8; females in excess of males 50/23, 49/38, 59/42, 23/7, 50/30, 63/24, 72/65. There is no regular seasonal change in the picture of general balance between the sexes. At Station 2 during March and April of 1933 more females than males were banded, viz., 325 and 296 respectively, but in 1934 and 1935, during the same two months, this was reversed, with 186 males and 108 females banded.

Field observations of spring and winter flocks, when differences in the appearance of the sexes make recognition certain, has provided no evidence of sexual segregation on migration, neither do they suggest any disproportion in numbers between the sexes as sometimes is apparent with other duck species; for example, the pintail.

Food and Feeding Habits

The food range of the green-winged teal is not extensive; seeds of aquatic plants, aquatic insects, molluscs, and crustaceans seem to be preferred in that order. Much food is obtained from the surface of ponds and shallow lakes.

In such places it is common to see these small ducks gliding here and there across the surface as minute objects are picked from the water, or pausing to submerge head and neck among the dense foliation of pond weeds or widgeon grass, where amphipods, insects, and seeds usually are plentiful. Foraging for similar foods in the rich vegetation of shoreward shallows, they tip up in the manner of all pond ducks, and so also they seek food of this sort along lake margins where wind-blown flotsam litters the shore. In shallow *Chara* meadows they seek out the oospores and avoid the branches that seem unattractive to them; apparently the leaf material of aquatic plants also seems unattractive. In winter, along the coast, flood ponds are visited in evening and morning following flights from the sea, and here they find weed seeds of many kinds.

Swarth (13) reports finding the eggs of humpback salmon, *Oncorhynchos gorbuscha*, in the gullets of green-winged teal collected on the Kispiox River, but the habit of feeding on salmon eggs has not been detected along the Pacific Coast.

The stomach contents of 55 adult green-winged teal collected in the Okanagan Valley, the Cariboo Parklands, and the Peace River Region of the interior, and 14 specimens from the mouth of the Fraser and from localities on Vancouver Island have been studied. The results in summary follow.

Food of Downy Young

The stomachs of two downy young, taken in June at Tupper Creek, Peace River region, contained insect material to the extent of 100% in one and 60% in the other, the food in largest bulk being the pupae of a midge (Ceratopogonidae); other insects represented were a midge larva, *Caoborus* sp., caddis larva, terrestrial coleopteran, and dragonfly nymph. Egg cases of *Cladocera* sp. were present in both stomachs and one contained a small amount of filamentous alga, *Zygnema.*

Food of Adults, Interior Region

All the stomachs are from specimens collected in September. A well-filled stomach from "Disputed Lake", near Horse Lake, in the Cariboo Parklands, contained the following insects: several dragonfly nymphs (Anisoptera), six damselfly nymphs (Zygoptera), 20 caddis-fly larvae (Phryganeidae), 70± mayfly nymphs (Ephemerida), and 10 chironomid larvae. Other animals present were: 1 leech, 100± small gastropods (*Planorbis* sp.), and 25± minute pelecopods. The plant material eaten included a quantity of water moss (*Fontinalis* sp.), and a few seeds each of *Potamogeton* sp., *Eleocharis* sp., *Sparganium* sp., and *Carex* sp. A small amount of a blue-green alga also was in the stomach. A specimen from Horse Lake contained seeds of *Scirpus acutus*, *Eleocharis palustris*, *Polygonum hydropiper*, *Carex* sp., and fragments of dragonfly nymphs. One from 103 Mile Lake held vegetable material only, including seeds of *Scirpus acutus*, *Potamogeton pusillus*, and *Carex* sp. In another from Tatton Lake, chironomid larvae constituted 80% of the contents, the balance being fragments of corixids, 100± seeds of *Scirpus acutus*, 20 seeds of *Potamogeton pusillus*, and one *Myriophyllum spicatum* seed. A few bryozoan statoblasts also were in the stomach. *Chara* oospores was the principal food eaten by three specimens collected at a meadow slough, near the north end of Okanagan Lake; in one, this item amounted to 100%, in the others, 70% and 60% of the total food. One had also eaten 38 seeds of *Scirpus acutus*, the other a small quantity of vegetable matter that was not identified.

The contents of 46 stomachs containing food collected at Swan Lake, Sept.—11, Oct.—26, Nov.—9, are summarized below.

Crustaceans. One stomach contained 8+ specimens of *Hyalella azteca*, constituting 20% of the contents; another 15% of *Gammarus limnaeus* debris; and traces of amphipods were noted in two others.

Insects. Aquatic insects were represented in 26 stomachs by amounts varying from less than 1%, to a maximum of 80%, of total contents. Corixidae were present in 13, *Chironomus* sp. larvae in one, Chironomidae larvae in four, and other insect groups as follows: Odonata—dragonfly nymphs 3, damselfly nymphs 2; Gyrinidae—larvae 3; *Dytiscus* sp.—larvae 3; terrestrial insects (adult Coleoptera) occurred twice and represented 40% of the total food in one, and 5% in another stomach. Insect debris in small amounts and not further identified was noted in seven specimens.

Molluscs. Minute *Physa* sp. and/or *Planorbis* sp. and mollusc debris occurred in nine stomachs, representing percentages of 75, 50, 40, 20, and minor items.

Miscellaneous animals. Leech egg cases, 100±, constituted 70% of the food in one stomach, and this was a minor item in two others; bryozoan statoblasts occurred in three, in one forming 24% of the food eaten.

Chara oospores. The oospores of *Chara* were present in 11 stomachs, and in six of them constituted 25 to 90% of the food present.

Scirpus seeds. The achenes of round-stem bulrush, *Scirpus acutus*, is the food item represented most often, and in the greatest volume. It occurred in 39 of the 46 stomachs examined, in 15 composing 90 to 100%, in 13 others 50 to 80%, and in the remainder from four to 36%, of the total food in the stomach.

Miscellaneous seeds. Seeds of aquatic plants other than *Scirpus acutus* occurred less frequently and in smaller amounts; the species and times of occurrence are: *Carex* sp. 1, *Eleocharis palustris* 2, *Sparganium* sp. 2, *Rumex maritimus* 3, *Zannichellia palustris* 2, *Polygonum amphibium* 3, *P. convolvulus* 1, *Myriophyllum spicatum* 3, *Potamogeton pectinatus* 4, *P. pusillus* 2, *P. heterophyllus* 3.

Miscellaneous vegetation. In this category are: one occurrence of winter buds of *Potamogeton pectinatus*; two of the root bulbils of *Chara*, in one instance composing 100% of the food in a partly filled stomach; one of nostoc; and one of an unidentified alga.

Food of Adults, Coast Region

The stomachs of 16 green-winged teal from the Coast Region have been examined; localities represented, numbers of specimens, and months in which they were taken are: Fraser River mouth (Ione Island, Sea Island, Lulu Island), Nov.—1, Dec.—2, Jan.—1; Boundary Bay, Oct.—4; Colquitz, Jan.—1; Cowichan Flats, Nov.—1, Jan.—2; Departure Bay, Jan.—1; Nanaimo Meadows, Jan.—1; Crofton Flats, Nov.—1. The last five localities are on Vancouver Island.

Crustaceans. There were three occurrences of marine amphipods from Sea Island, Boundary Bay, and Nanaimo Meadows, in one constituting 40, in another 25% of the stomach contents.

Molluscs. Small marine gastropods represented 50% of the contents of a specimen from Departure Bay.

Insects. Adult diptera were present in two from Boundary Bay, in volume percentage of 40 and 50 respectively. Both of these ducks and one from Cowichan Flats had eaten terrestrial coleopterans. In another were two larvae of an aquatic beetle. Chironomid larvae was the largest food item in a specimen from Nanaimo Meadows.

Miscellaneous animals. Here are placed one occurrence of polychaete jaws present in a stomach from Boundary Bay; and an occurrence of unidentified fish eggs that composed 80% of the contents in a Cowichan Bay specimen.

Scirpus seed. The seeds of a three-square bulrush, *Scirpus americanus*, was the chief food in three specimens from the Fraser River mouth and represented 40% of the food in another. Elsewhere it was recorded only from the Cowichan Flats and as a minor percentage.

Miscellaneous seeds. Thirteen specimens contained seeds, other than *Scirpus*, and composed 50% of the total volume in a specimen from Departure Bay. *Carex* species occurred five times in varying amounts. *Eleocharis palustris* occurred four times, volume percentage up to 25; *Polygonum amphibium* occurred twice, *P. lapathifolium* and an unidentified polygonum occurred once each. Seeds of other plants, which occurred in small amounts, are: *Sparganium* sp., *Bromus* sp., *Atriplex* sp., *Distichlis patula*, and *Salicornia ambigua*.

Food Summary

One of two downy young from the Peace River District had eaten insects exclusively, the other to the extent of 60% of the stomach contents. The chief constituent in each was a midge pupa. In 53 stomachs of adults from the interior vegetable matter constituted 66% and animal matter 34% of the total food eaten. As indicated by frequency of occurrence preference was shown for various foods in this order: seeds of aquatic plants, including 45 occurrences of *Scirpus acutus*; insects including Odonata nymphs, chironomid adults and larvae, corixids, and coleopteran adults and larvae; *Chara*

TABLE VIII

FREQUENCY OCCURRENCE OF IDENTIFIED FOOD ITEMS IN 69 GREEN-WINGED TEAL STOMACHS

Waterflea, *Cladocera*	Egg cases	1
Amphipod, unidentified marine form		3
Amphipod, unidentified freshwater form		1
Amphipod, *Hyalella azteca*		1
Amphipod, *Gammarus limnaeus*		1
Leech (Hirudinea)	Egg cases	2
Leech (Hirudinea)	Adult	1
Damselflies, *Enallagma* spp.	Nymph	3
Dragonflies (Odonata)	Nymph	4
Water boatman (Corixidae)	Adult	14
Caddis flies (Tricoptera)	Larva	2
Beetle, terrestrial	Adult	8
Beetle (Gyrinidae)	Adult	1
Beetle (Dytiscidae)	Larva	2
Beetle, aquatic	Larva	5
Beetle, *Haliplus* sp.	Adult	2
Beetle, *Haliplus* sp.	Larva	1
Midge (Chironomidae)	Adult	2
Midge (Chirobomidae)	Larva	3
Midge (Ceratopogonidae)	Pupa	2
Midge, *Caobornis* sp.	Larva	1
Fly (Diptera)	Adult	2
Mussel, *Pisidium* sp.		1
Snail, unidentified freshwater form		6
Snail, unidentified marine form		1
Snail, *Planorbis* sp.		4
Snail, *Physa* sp.		1
Marine worm (Polychaeta)		1
Bryozoa	Statoblasts	4
Muskgrass, *Chara* sp.	Oospores	14
Muskgrass, *Chara* sp.	Bulbils	2
Algae, unidentified freshwater forms		3
Filamentous alga, *Zygnema*		1
Water moss, *Fontinalis* sp.		1
Bur-reed, *Sparganium* sp.	Seeds	3
Pondweeds, *Potamogeton* sp.	Seeds	3
Potamogeton heterophyllus	Seeds	3
Potamogeton pusillus	Seeds	4
Potamogeton pectinatus	Seeds	4
Potamogeton pectinatus	Winter buds	1
Horned pondweed, *Zannichellia palustris*	Seeds	2
Salt grass, *Distichlis patula*	Seeds	2
Brome grass, *Bromus* sp.	Seeds	2
Sedge, *Carex* sp.	Seeds	11
Bulrush, *Scirpus acutus*	Seeds	45
Bulrush, *Scirpus americanus*	Seeds	3
Spike rush, *Eleocharis* sp.	Seeds	2
Eleocharis palustris	Seeds	6
Dock, *Rumex maritimus*	Seeds	4
Bindweed, *Polygonum Convolvulus*	Seeds	1
Smartweed, *Polygonum amphibium*	Seeds	6
Polygonum lapathifolium	Seeds	1
Polygonum Hydropiper	Seeds	1
Glasswort, *Salicornia ambigua*	Seeds	1
Orach, *Atriplex* sp.	Seeds	3
Water milfoil, *Myriophyllum spicatum*	Seeds	4

oospores, molluscs, and crustaceans. In 16 stomachs from the coast, vegetable matter constituted 67% and animal matter 33% of the total contents. Seeds of aquatic and shore plants were first in importance followed by insects, crustaceans, and molluscs in that order.

TABLE IX

Food of 69 adult green-winged teal, percentage of total volume

Localities and number of specimens		Crustaceans	Insects	Molluscs	Misc. animals	*Chara* oospores	*Scirpus* seeds	Misc. seeds	Misc. vegetation
Tatton Lake	1		81.00		1.00		15.00	3.00	
103 Mile Lake	1						1.00	39.00	60.00
Horse Lake	1		30.00				30.00	40.00	
"Disputed" Lake	1		55.00	15.00	5.00			15.00	10.00
Pond, Okanagan	3					76.66	13.34		10.00
Swan Lake	46	1.09	11.72	4.65	2.11	7.17	50.66	12.59	10.01
Mouth Fraser River	4	27.50					51.50	11.00	10.00
Boundary Bay	4	6.25	63.00		10.00			2.50	18.25
Colquitz, V.I.	1							100.00	
Crofton, V.I.	1							100.00	
Cowichan Flats, V.I.	3				26.66		3.34	70.00	
Nanaimo Meadows, V.I.	2	2.50	45.00					50.00	2.50
Departure Bay, V.I.	1			50.00				50.00	

Economic Status

Unlike certain other duck species the green-winged teal has exhibited no tendency to feed on agricultural crops, neither do its habits conflict otherwise with human interests. In this respect then its economic status is neutral. The value of the waterfowl resource in terms of national economy was noted in earlier papers of this series and it need only be mentioned here that the green-winged teal constitutes an important element in this resource. It stands high on the list of table birds and is a game duck of distinction—the more discerning appreciating the quality of speed in flight that tests the hunter's skill.

Acknowledgments

During the course of this study laboratory facilities were provided by Dr. R. E. Foerster, Director, Pacific Biological Station, and by Dr. G. Clifford Carl, Director, British Columbia Provincial Museum. Dr. I. McT. Cowan, Mr. Kenneth Racey, and Mr. Robert Luscher provided some of the green-winged teal stomachs reported on, and from the British Columbia Game Commission many of the banding data were obtained. In acknowledging this helpful co-operation I wish to extend my grateful thanks.

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THE EFFECT OF VITAMIN A ON HEREDITARY HYPERKERATOSIS IN THE MOUSE[1]

BY F. CLARKE FRASER

Abstract

The formation of cysts in the sebaceous glands and follicle ends that occurs in "rhino" mice can be suppressed by massive vitamin A therapy. The significance of this finding is discussed in relation to the mode of action of the rhino gene.

Introduction

It was shown in previous communications (12, 2) that the simple recessive "rhino" (hr^{rh}) gene in the mouse causes a follicular hyperkeratosis associated with depilation and subsequent cyst formation in the hair follicles and sebaceous glands. Transplantation experiments showed that rhino skin when grafted at birth to a normal host would behave autonomously in the center of the graft, but that around the edges of the graft, where rhino skin grew in close proximity to normal skin, the genetically rhino follicles behaved like normal follicles and did not lose their hair or undergo cyst formation. This suggested that there is a cell-diffusible substance produced by normal skin cells but not present in the blood stream that is necessary for the normal maintenance of the cutaneous epithelium. Skin cells homozygous for the rhino gene presumably cannot produce this substance in quantities sufficient to keep the skin normal, although apparently they can utilize it when it is supplied by adjacent normal skin cells.

Because of the histological similarities between rhino skin and that of animals fed diets deficient in vitamin A (3, 7, 4, 9, 13), it was thought that the substance postulated to be deficient in rhino skin might be a metabolite of vitamin A. If this is so, then increasing the blood concentration of vitamin A in rhino mice might lead to an increased production of the substance and cause a regression of the rhino condition towards normal.

A similar problem exists clinically, with regard to the treatment of several types of hereditary hyperkeratoses with vitamin A (10, 11, 5). The high degree of variability between patients in blood levels of vitamin A, dark adaptation, and response to treatment with vitamin A in cases of pityriasis rubra and Darier's disease (two hereditary diseases involving a cutaneous hyperkeratosis) may be accounted for on the assumption that in different

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cases different genes may be involved, each interfering with the vitamin A metabolism at a different point. The skin may manifest a lack of vitamin A because it is deficient in the blood (due to impaired absorption or liver function) or because it cannot be utilized by the skin cell. In view of the experiments about to be reported, it is thought that the rhino mouse may serve as an experimental medium for investigation of the latter possibility.

Experiments previously reported (2) in which rhino mice were fed massive doses of vitamin A in the form of ling liver oil gave results that were rendered inconclusive by the systemic toxicity of the treatment. The question of whether this toxicity was associated with the vitamin A or was entirely due to some other constituent in the preparation has been answered by the demonstration of Moore and Wang (8) that crystalline vitamin A is itself toxic.

Experimental Results

(a) *Systemic Toxicity*

The mice in this experiment were fed a highly purified distillate of the natural esters of vitamin A containing 500,000 International Units of vitamin A per gram, suitably diluted with Mazola oil and administered orally every other day through a graduated medicine dropper. Control litter mates were fed equivalent amounts of Mazola oil in a similar way.

Throughout the experiments the dosage was maintained at a maximum compatible with the general health of the mouse as estimated by the absence of any of the outward signs of systemic toxicity discussed below. Doses of the order of 5000 I.U. every other day for about one month to adult rhino mice caused the skin to become hyperemic, oily, and covered with fine powdery scales. The mice at this time became restless, easily excited, and scratched themselves a good deal as if there was some cutaneous irritation. If treatment was continued there was a rapid loss of weight and the animals became listless, emaciated, and eventually moribund within two weeks of the appearance of toxic signs. The only consistent macroscopically noticeable post-mortem findings other than the cutaneous effects described below were a marked enlargement of the spleen and a less pronounced enlargement of the liver. The degree of enlargement of these organs varied with the dosage and the length of time the animals survived treatment. Associated histological changes are described in Section *b* (1). Discrepancies between these findings and those described in other reports of systemic vitamin A toxicity (14, 6, 8) may be due to the more chronic nature of the treatment in the present case. This aspect of the problem will be investigated more fully.

The dosage level that will permit administration over long periods without external signs of systemic toxicity has been established as being of the order of 500 to 1000 I.U. every other day. Although enlargement of the liver and spleen does occur at this dosage level, several mice have survived for as long as eight months at this dosage without showing any outward signs of the treatment except the skin changes about to be described.

(b) *Skin Effects*

(1) *Feeding Vitamin A*

Experimental results indicate that a high blood level of vitamin A is associated with inhibition of the growth of the sebaceous and follicle-end cysts that ordinarily develop in rhino skin following hair loss. The records of one pair of mice will be presented in some detail to demonstrate this characteristic response to treatment. A rhino male, 2804, was fed vitamin A concentrate beginning at an age of 17 days with a dose of 1250 I.U. every other day, increased to 5000 I.U. every other day at five weeks. Male 2807, a rhino litter-mate of this mouse was used as a control. At eight weeks of age the skin of the treated animal was noticeably pinker, more oily, thinner, and smoother than that of the control, which had begun to develop the characteristic rhino thickening and wrinkling (Figs. 1 and 2). In another week the skin of the treated mouse was very pink, and, in the dorsal shoulder region, covered with fine powdery white flakes. The mouse was breathing rapidly and the liver, which could be seen through the skin, was noticeably enlarged. A similarly treated litter-mate, after a rapid loss of weight, died at this time. Consequently the dose was reduced to 1250 I.U. every other day until the age of 21 weeks, when the skin of the treated animal was still soft, thin and smooth, although that of the control continued to get thicker and more wrinkled. Here the dose of vitamin A was reduced to about 600 I.U. every other day and continued at this level until death of the animal at 50 weeks. At death the spleen of the treated animal was tremendously enlarged, weighing 0.7 gm. as compared with a weight of 0.1 gm. for the spleen of the control. The organ appeared congested with blood but was very firm and the cut surface showed many dense white nodules. The liver was also enlarged but not as much as the spleen. Its surface was speckled with white spots and there were several hemorrhagic areas visible.

I am indebted to Dr. W. E. Finkelstein of the Department of Pathology, McGill University, for the following histopathological observations on these organs, and hereby extend my thanks to him for his generous co-operation in this respect. "One section of liver from male 2804 is examined. Sections are stained with Masson's trichrome, Masson's hemalum, phloxine and saffron, Weigert's elastic tissue and Wilder's reticulum stain. There is severe alteration, distortion and loss of the normal hepatic architecture. Broad interlacing bundles of moderately stout collagen fibres forming a loose network intersect throughout the section, enclosing larger and smaller irregularly outlined areas of surviving parenchyma. The spaces in this collagenous network are heavily infiltrated with large numbers of both acute and chronic inflammatory cells. Polymorphonuclear leukocytes and large mononuclear cells are present in approximately equal numbers. These are large, round to oval with prominent, round, oval or reniform nuclei. One of the larger portal veins shows marked cellular infiltration of its wall by polymorphonuclear cells and macrophages. The bands of fibrosis include the portal tract and apparently also incorporate the central veins, although it is difficult to distinguish with certainty between portal and central veins. There is minimal proliferation of bile ducts in the portal tracts and they are largely obscured by the intensity of the surrounding inflammatory reaction. In several instances, the lining cells of the bile ducts show small brightly acidophilic droplets in their cytoplasm. At the borders of the bands of fibrous tissue collagenous fibres insinuate themselves for short distances between liver cells and in some places, the cells so enclosed are compressed, shrunken and in many instances necrotic. Similar hepatic cells are seen to be sequestered in the central portions of some of the zones of fibrosis. The surviving hepatic parenchyma shows loss of the usual radial pattern and the cells exhibit mild degenerative changes. Focal areas of necrosis are present throughout the surviving parenchyma often bordering on the bands of fibrous tissue but occasionally seen remote from them. In such areas, the liver cells are compressed and atrophic with strongly acidophilic cytoplasm; many show karyorrhexis and karyolysis. Occasional mitonecroses are noted. In some of these areas, the liver cells have completely disappeared, only a collapsed reticulin network remaining. A few of the liver cells show fine cytoplasmic vacuolation but this change is rare and not conspicuous. Sections of liver stained for bacteria by Glynn's method and with Ziehl-Nielsen carbol-fuchsin for acid-fast organisms are negative.

"In summary, the section examined shows severe degenerative, inflammatory and productive changes. The histological pattern of bands of fibrous tissue enclosing larger and smaller masses of persistent hepatic parenchyme is indistinguishable from that seen in active cirrhosis.

"One section of spleen from male 2804, stained with Masson's hemalum, phloxine and saffron is examined. The spleen is hyperemic and the sinusoids are filled with red cells. Lymphoid follicles are small and reduced in number. The red pulp is highly cellular being packed with numerous lymphoid and reticular elements. Many of the cells are lymphocytes while numerous large mononuclear cells are present. Occasional mitotic figures are present, polymorphonuclear leukocytes are present in moderate numbers and megakaryocytes are also present. The alteration in the spleen is non-specific in character and may be characterized as hyperplasia of the pulp."

The lungs were congested and showed many petechial hemorrhages. No pathological lesions were found in the control animal.

Biopsy specimens taken at 31 weeks showed that in the skin of the treated mouse there was an almost complete absence of cysts in the sebaceous glands and follicle ends, although there were many hair canal cysts present. Sections of skin removed at death (50 weeks) from the treated animal showed that the only change that had occurred since 31 weeks was that the hair canal cysts

FIG. 1. *Male 2807, untreated rhino, eight weeks old.*
FIG. 2. *Male 2804, rhino treated with vitamin A, same age.*
FIG. 3. *Skin from rhino male 2807, 50 weeks old.* ×85.
FIG. 4. *Skin from rhino male 2804, same age, treated with vitamin A.* ×85.

had become smaller – as they had also in the control – and the connective tissue of the corium had become a little denser (Figs. 3 and 4). In the control animal, on the other hand, the sebaceous and follicle-end cysts had become much larger during this interval.

The vitamin A treatment does not seem to cause much, if any, actual regression of the sebaceous and follicle-end cysts, it merely stops them from developing any further. This conclusion was drawn from two animals in which treatment was started at 10 weeks of age, well after cyst formation had begun. Biopsy specimens taken at the beginning of treatment and after 19 weeks of treatment showed that the degree of cyst formation was about the same in the treated animal before and after treatment, but had progressed noticeably in the control animals over the same period.

This inhibitory effect of the treatment on cyst formation is a regular phenomenon, which has been observed, at various stages, in a total of 15 rhino mice, each one compared with an untreated rhino litter-mate. No such inhibition has ever been seen in the absence of treatment in any of the hundreds of rhino animals observed by the author, and it seems clearly established that the effect is a consequence of treatment.

So far no animal in which treatment was started before the time of hair loss has survived. In four rhino mice that were fed 300 I.U. of vitamin A every other day starting at three days of age and that died on the 30th day, no effect of treatment on the rate of hair loss was noted.

Two nonrhino animals from the rhino strain (which is maintained by breeding rhino males with heterozygous sisters) were fed vitamin A starting at four weeks of age with doses of 1250 I.U. every other day. This was increased to 4000 I.U. every other day at six months, and maintained here till the age of seven months. Throughout this period no effect on the outward appearance of the hair coat was observed, nor did histological examination show any difference between the skins of the treated mice and those of control nonrhino litter-mates.

(2) *Feeding Carotene*

No significant changes were observed in the skins of two rhino mice fed 2500 I.U. of carotene every other day for 10 weeks beginning at five weeks of age. Nor were there any signs of systemic toxicity noticed during treatment or at autopsy. Control litter-mates were fed corresponding amounts of Mazola oil.

(3) *Feeding Vitamins A and C*

Since the early signs of vitamin A intoxication resemble those occurring in scurvy (8, 14), the effect of feeding vitamin C to mice being treated with vitamin A was observed. Two rhino members of a litter were fed 1250 I.U. of vitamin A every other day beginning at the age of four weeks. Two other rhino animals from the same litter were fed similar doses of vitamin A, with 1 mgm. of vitamin C in aqueous solution being given concurrently. No effect of the vitamin C on either the cutaneous signs of vitamin A treatment or the enlargement of liver and spleen could be detected after two months of treatment. This result is in agreement with those of Moore and Wang (8) who performed similar experiments in rats.

Discussion

Although the above experiments were largely of an exploratory nature, they have clearly demonstrated that a high blood level of vitamin A is associated with suppression of the process leading to cyst formation in the sebaceous glands and follicle ends in the rhino mouse. Whether this effect is due to the fact that the treatment supplies the rhino skin with some metabolite of vitamin A that is necessary for maintenance of the skin in a normal condition is not entirely proved. The possibility that the retardation in cyst formation is a secondary manifestation of the systemic toxicity has not been excluded. However, the fact that a similar dosage of vitamin A in nonrhino mice does not seem to retard hair growth argues against this interpretation. The question of why the treatment affects cyst formation in the sebaceous glands and follicle ends and not in the hair canal also remains unanswered. Perhaps the dosage of vitamin A necessary to affect the formation of hair canal cysts is above the level of toxicity, and kills the mouse before it is able to cure the condition.

In conclusion, it can be stated that the experimental results reported above are compatible with the assumption that the presence of the rhino gene in the homozygous condition is associated with a decreased ability of the skin cells to utilize some metabolite of vitamin A necessary for their normal maintenance. Enzymatic studies on the utilization of vitamin A by rhino and nonrhino skin respectively should provide further relevant information. If the gene does act by causing a metabolic deficiency analogous to those studied so profitably in the lower organisms (1), it might provide a useful tool for the analysis of the intracellular metabolism of vitamin A.

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SOME EFFECTS OF P^{32} ON THE DEVELOPMENT OF *DROSOPHILA*[1]

By T. J. Arnason,[2] R. L. Irwin,[3] and J. W. T. Spinks[4]

Abstract

When freshly laid *Drosophila* eggs were placed in a graded series of P^{32} concentrations in the culture media, death of treated individuals occurred most frequently at the end of the larval period and in the pupal stages. No adults emerged from cultures having an initial concentration of 0.65 rutherford (rd.) or higher per ml. food. The calculated concentration of P^{32} expected to reduce emergence of adults to 50% is 0.120 rd. per ml. A dosage of 1.30 rd. per ml. prevents transformation of larvae to pupae. Three out of 132 X-chromosomes from males reared in medium having an initial concentration of 0.0325 rd. per ml. carried new recessive lethal "genes" .

Introduction

In planning experiments designed to reveal the mutagenic efficiency of radioisotopes, it is desirable to know the tolerance limit of the organism for the isotopes since maximal mutation frequency may be expected at the highest applied dosages. *Drosophila melanogaster* appears to be a particularly suitable organism for testing since exact determinations of the frequency of X-ray induced mutations have been made repeatedly (2). It is thought, therefore, that the establishment of the minimal dose of radiophosphorus that is lethal to *Drosophila* will be of some interest.

Materials and Methods

Eggs of wild type (Canton Special Stock) were collected by means of a 5 ml. shell vial apparatus, illustrated in Fig. 1. Twelve to fourteen females and

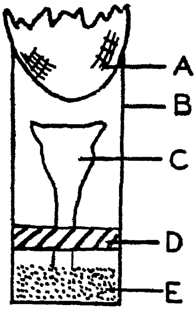

Fig. 1. *Vial for collection of eggs. A, cotton and gauze plug; B, shell vial, 5 ml.; C, filter paper wick; D, cork slice; E, banana–yeast mixture.*

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several males were placed in the vial where they were able to feed on the juice of a banana and yeast mixture, drawn by capillarity from the lower part of the vial. Flies were kept for 24 to 48 hr. in the vials prior to egg collection. After this period of acclimatization the flies appeared to be stimulated in egg production.

The eggs were laid on the cork, which had been lightly smeared with banana pulp. The eggs were removed by means of fine steel needles and transferred to the surface of the medium, containing radiophosphorus, in a 5 ml. shell vial. All eggs used were collected within eight hours of laying.

The culture medium employed was the usual (7) corn meal, agar, molasses, and yeast mixture. Radiophosphorus, in the form of phosphoric acid was mixed with the medium, the total volume of medium plus P^{32} being one ml. per vial. Nine series of vials, labelled I to IX, were used, each series consisting of five vials containing concentrations of P^{32} as listed in Table I. Fifteen eggs were placed in each vial. The vials were placed in an incubator kept at 23° ± 2° C. and a high humidity (to prevent drying of the cultures).

TABLE I

CONCENTRATIONS OF P^{32} USED, RD. PER VIAL*

Vial	A	B	C	D	E
P^{32} activity, rd.	0	0.065	0.325	0.65	1.3

* *We are grateful to W. D. Murray for radioactivity measurements.*

All the larvae from Series I to VI were extracted after 2.5, 5, 7, 9, 10, and 12 day periods respectively. Series VII to IX were allowed to reach the adult stage and the adults were used for emergence and breeding tests.

The extracted larvae from Series I to VI were washed twice with water and once with 70% alcohol after which they were placed on a cover slip and warmed over a lamp with a drop of 3 : 1 alcohol – acetic acid mixture. This latter process killed, expanded, and fixed the larva. An ocular micrometer in a binocular microscope was used in measuring larval lengths.

The amount of P^{32} in a specimen was measured by placing the cover slip, on which the larva was fixed, in a reproducible position under the window of an end-on Geiger-Müller chamber (mica window, 3 mgm. per sq. cm.) and counting it. The chamber was connected to a scale of 64 scaling circuit and any sample was counted for a sufficient length of time to give approximately 10,000 counts (standard deviation is then about 1%). By placing the cover slip at a suitable distance below the counter window, the rate of counting was kept below 2000 counts per minute and the correction for resolving time of the tube (2×10^{-4} sec.) was less than 1%.

The natural rate of the counting tube (so-called "background") was allowed for by subtracting it from the total counting rate for any sample. The radioactive decay of P^{32} (half life 14.3 days) was allowed for by taking an aliquot of the original stock solution, evaporating it to dryness, and counting it immediately before and after counting the unknown material. The aliquot of the original thus serves as a standard and eliminates the necessity of correcting for the decay of the P^{32} or for small fluctuations in the counter. In some experiments, counter fluctuations were allowed for by comparing the unknown with a uranium oxide standard. Absolute rates of disintegration were obtained by multiplying the observed counting rate by a geometry factor obtained by calibrating the counter with an RaD + E standard from the Bureau of Standards (8).

The weight of the larvae ($<$ 1.2 mgm.) is such that self absorption corrections are negligible (self absorption half thickness for P^{32} radiations is approximately 300 mgm. per sq. cm.) and it is thus not necessary to ash the sample before counting.

As flies from Series VII to IX emerged, both sex and time of emergence were recorded and individuals to be used for breeding were transferred immediately. Some adults were dissected to test for radioactivity in various parts of the body. Twenty-six days after the treatment began it was judged that no further emergence could be expected and all remaining pupae and puparia were extracted. Their radioactivity was measured and the amount of pupal differentiation noted. An arbitrary measurement of the amount of development was used.

Stage 0 : larval development only.
Stage 1 : puparia formation but no development of gross features.
Stage 2 : differentiation of gross features (head, thorax, abdomen).
Stage 3 : development of gross features but incomplete differentiation of minute structures.
Stage 4 : full development, no emergence.
Stage 5 : full development and emergence.

Results

1. *Measurement of Radioactivity and Development in Larvae*

The measurements of larval length and total contained activity are recorded graphically in Figs. 2 to 5. It appears that, for all initial concentrations, the larger the larva, the greater the amount of radioactivity contained.

The variation of average larval length with time and initial radioactivity is recorded in Table II. From the results it is apparent that the P^{32} of these concentrations had little noticeable effect on the growth of the surviving larvae.

2. *Measurement of Radioactivity and Development in Pupae*

The amount of radioactivity in pupae that had failed to emerge by the 26th day from the start of treatment is recorded in Table III. In the B concentration (not recorded in the table), only three pupae had failed to emerge.

The amount of P^{32} in pupae is roughly proportional to the concentration in the medium. Differentiation of pupae is decreased as the concentration of radioactivity rises.

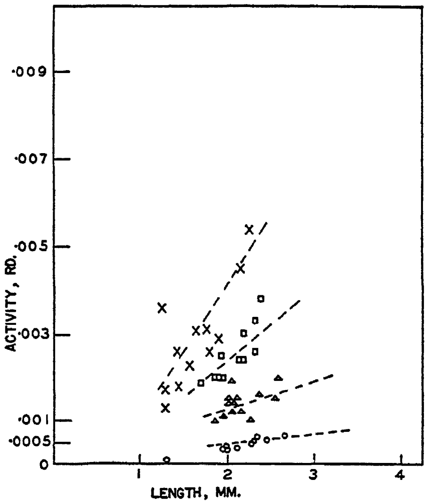

FIG. 2.

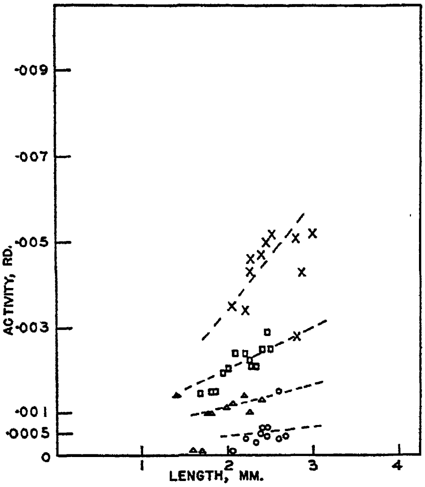

FIG. 3.

FIG. 2. *Larval length at five days, plotted against contained P^{32} activity in rd. Original concentration P^{32} in medium in rd. per ml.* ○, *0.065;* △, *0.325;* □, *0.65;* ×, *1.3.*

FIG. 3. *Larval length at seven days, plotted against contained P^{32} activity in rd. Original concentration P^{32} in medium in rd. per ml.* ○, *0.065;* △, *0.325;* □, *0.65;* ×, *1.3.*

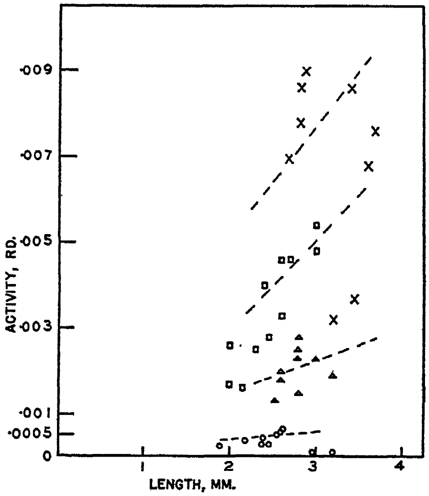

FIG. 4.

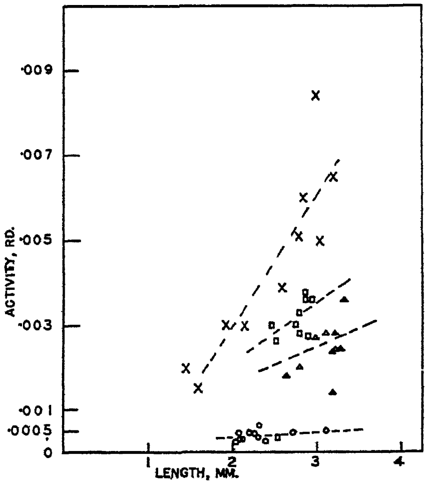

FIG. 5.

FIG. 4. *Larval length at nine days, plotted against contained P^{32} activity in rd. Original concentration P^{32} in medium in rd. per ml.* ○, *0.065;* △, *0.325;* □, *0.65;* ×, *1.3.*

FIG. 5. *Larval length at 10 days, plotted against contained P^{32} activity in rd. Original concentration P^{32} in medium in rd. per ml.* ○, *0.065;* △, *0.325;* □, *0.65;* ×, *1.3.*

TABLE II

P^{32} EFFECTS ON GROWTH OF LARVAE

Initial activity P^{32}, rd./ml.	Average larval length, mm. The number of larvae extracted from each vial, in brackets				
	2.5 days	5 days	7 days	10 days	12 days
0	1.83 (15)	2.27 (12)	2.46 (13)	2.81 (12)	3.02 (11)
0.065	1.21 (14)	2.01 (12)	2.51 (10)	2.47 (13)	2.31 (13)
0.325	1.44 (13)	2.15 (13)	1.92 (13)	2.87 (13)	3.09 (11)
0.65	1.20 (12)	1.87 (12)	2.12 (14)	2.51 (13)	2.74 (10)
1.3	1.18 (13)	1.67 (13)	2.47 (10)	2.69 (8)	2.46 (10)

TABLE III

DEGREE OF DEVELOPMENT AND AMOUNT OF RADIOACTIVITY IN INDIVIDUAL PUPAE (AT 26 DAYS)

Concentration C (0.325 rd. per ml.)		Concentration D (0.65 rd. per ml.)		Concentration E (1.3 rd. per ml.)	
Development of pupa, stage	Total radioactivity in pupa and puparium, rd.	Development of pupa, stage	Total radioactivity in pupa and puparium, rd.	Development of pupa, stage	Total radioactivity in pupa and puparium, rd.
4	0.00023	1	0.00080	0	0.00090
4	0.00047	1	0.00077	0	0.00170
4	0.00036	1	0.00106	0	0.00530
3	0.00038	2	0.00046	0	0.00320
3	0.00033	1	0.00098	0	0.00540
				0	0.00100
				1	0.00098
Average	0.00035	—	0.00081	—	0.00264

3. *Measurement of Radioactivity in Whole and Dissected Adults*

Activity measurements were made on 10 flies reared on medium of B concentration. Three of the flies emerged from pupae that had been transferred to a medium containing no activity. Recorded activities in these three were as high as in flies that emerged in vials containing P^{32}.

The average percentages of the total radioactivity appearing in the main divisions of the body were as follows: head, 16%; thorax including appendages, 47%; abdomen, 37%. Less than 5% of the activity was in the wings and legs. The results are recorded in Table IV.

4. *Pupation*

All the individuals developing in Series VII to IX were grouped together for observations on pupal differentiation and emergence of adults. The numbers of pupae developing in the various concentrations of radioactivity are recorded in Table V. At the highest activity very few larvae managed to pupate. The percentage of pupae formed as a percentage of the eggs seeded rises as activity concentration declines.

TABLE IV

P^{32} ACTIVITY, IN RD., IN YOUNG ADULT *Drosophila* REARED ON MEDIUM CONTAINING 0.065 RD. PER ML.

Fly number	Head	Thorax	Wings	Legs	Abdomen	Total	Fly number	Whole fly
1	0.000057	0.000148	0.000000	0.000009	0.000126	0.000340	6	0.000288
2	0.000060	0.000107	0.000007	0.000012	0.000176	0.000362	7	0.000325
3	0.000052	0.000172	0.000013	0.000005	0.000128	0.000370	8*	0.000324
4	0.000051	0.000140	0.000002	0.000022	0.000114	0.000329	9*	0.000278
5	0.000055	0.000177	0.000001	0.000013	0.000097	0.000343	10*	0.000423
Average	0.000055	0.000149	0.000005	0.000012	0.000128	0.000349	—	0.000328

* *Transferred to inactive medium before emergence of adults.*

TABLE V

NUMBERS OF PUPAE FORMED FOR VARIOUS INITIAL ACTIVITIES OF RADIOPHOSPHORUS

Initial activity, rd.	0	0.065	0.325	0.65	1.3
Number of eggs seeded	45	45	45	45	45
Number of pupae formed	38	31	27	17	3
Percentage of pupae formed as percentage of eggs seeded	84	69	60	38	7

5. *Inhibition of Pupal Development in High P^{32} Concentration*

The number and the average degree of development of the pupae remaining in the 26-day old cultures of Series VII to IX is recorded in Table VI. By this time adults had already emerged in the A, B, and C concentrations as is reported in Table VII. Very few pupae were formed in Concentration E, and little pupal differentiation occurred in D.

TABLE VI

PUPAL DEVELOPMENT FOR DIFFERENT INITIAL ACTIVITIES OF P^{32}

Initial activity P^{32}, rd. per ml.	0	0.065	0.0325	0.65	1.3
No. of pupae remaining in 26-day old culture	1	3	24	17	3
Average stage of development of pupae remaining	4	3–4	2–3	1	0–1

6. *Emergence*

Results of emergence for Groups A, B, C, D, E in Series VII to IX are listed in Table VII. At concentrations of 0.65 rd. of P^{32} or higher, no adults emerged; at the 0.325 rd. concentration 6.6% emergence was recorded but all the flies died within three days. However, at 0.065 rd., the next lower concentration, 62.2% of the treated individuals emerged.

TABLE VII

NUMBER OF FLIES EMERGING FROM DIFFERING INITIAL ACTIVITIES OF P^{32}

Initial activity of P^{32}, rd. per ml.	Number of flies emerging														
	0			0.065			0.325			0.65			1.3		
Time of emergence, days	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total
15	7	12	19	2	4	6	1*	0	1	0	0	0	0	0	0
17	10	5	34	1	10	17	0	0	1	0	0	0	0	0	0
19	0	2	36	0	2	19	2*	0	3	0	0	0	0	0	0
20	0	1	37	2	5	26	0	0	3	0	0	0	0	0	0
22	0	0	37	0	2	28	0	0	3	0	0	0	0	0	0
26	0	0	37	0	0	28	0	0	3	0	0	0	0	0	0
No. of eggs	45			45			45			45			45		
% Emergence	82.2			62.2			6.6			0			0		

* *Died three days after emergence.*

7. *Lethal Mutations**

Wild-type larvae of the Canton Special stock, which has a low spontaneous mutation rate, were reared in culture medium containing 0.0325 rd. P^{32} per ml. Forty-four treated males were successfully mated to C 1 B females. Three of 132 C 1 B daughters from these crosses gave aberrant sex ratio as follows:

95 ♀♀ : 0 ♂; 51 ♀♀ : 0 ♂; 28 ♀♀ : 1 ♂.

It is concluded that two of 132 tested X-chromosomes had new recessive lethals; in a third X-chromosome, a semilethal mutation may have occurred. The observed proportion of male gametes carrying new recessive lethal mutations is 0.015 or 0.023, depending on whether the marginal lethal is counted or not. The mutation rate for sex-linked recessive lethals from sperms irradiated with 1000 *r* of X rays is 0.0289 (2).

Discussion

Radiophosphorus supplied to *Drosophila* in the food medium for the whole of the developmental period obviously does not have an immediate lethal effect on larvae at any of the concentrations used. Above a certain level, the cumulative effect of continued radiation kills old larvae, pupae, and adults. Our estimates of lethal effects are based on determinations of the initial concentration of P^{32} in the food medium required to kill individuals prior to emergence of adults. On this basis a dosage of 0.65 rd. per ml. is completely lethal and the calculated mid-lethal dose is 0.120 rd. per ml. (Fig. 6).

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* *We are indebted to Hulda Regehr for these observations.*

Most of the deaths occurred in late larval and in pupal stages. This does not necessarily imply greater sensitivity at these stages, since dosage effects are cumulative and these (older) individuals have absorbed more r.e.p.* than have the (younger) larvae. Mavor (3) found that a much smaller dosage of X rays was required to kill eggs, larvae, and young pupae than was needed for older individuals.

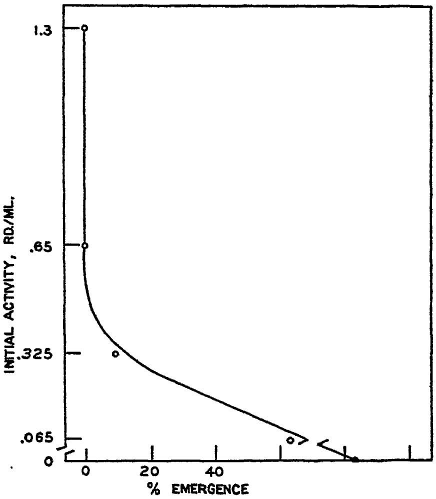

FIG. 6. *Percentage of emergence versus initial activity in rd. per ml.*

The studies on pupal differentiation indicate that the amount of pupal development is roughly in inverse ratio to the P^{32} dosage. The failure to differentiate may result from effects on earlier larval stages as well as from direct effects of radiations on the pupae. When pupation fails to occur one of the possible mechanisms is by radiation effects on the ring gland whose hormone is known to influence pupation (1, 5).

It is probable that radiation effects include disruption of enzyme systems and inhibition of various syntheses necessary for pupal differentiation. Altered cell division rates may also be important, particularly in the pupal stages. Normally, according to Poulson (6), few divisions occur in larvae more than 12 hr. old, and larvae younger than that have received relatively little radiation even at the highest dosage used.

Acknowledgments

We are indebted to the National Research Council of Canada for support of this work. We are grateful also to the Carnegie Institution of Washington for supplying stocks of *Drosophila*.

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* *Roentgen-equivalents-physical (4).*

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THE PROVENTRICULAR REGION OF *TETRAOPES TETRAOPHTHALMUS* FORST. (COLEOPTERA: CERAMBYCIDAE)[1]

By W. W. Judd[2]

Abstract

The proventricular region of *Tetraopes tetraophthalmus* Forst. is relatively poorly developed, consisting merely of the posterior end of the stomodaeum, which tapers toward the mid-gut and is invaginated into the anterior end of the mid-gut to form the oesophageal valve. It is of the type found in inactive insects that feed on plants and in which the crop is small.

Materials and Methods

Adult specimens of the milkweed beetle, *Tetraopes tetraophthalmus* Forst., were collected from the leaves and stems of the common milkweed, *Asclepias syriaca* L., growing along roadsides and in fields adjacent to the campus of McMaster University, Hamilton, Ont., Aug. 1 and 2, 1947. They were identified with a key to the genus *Tetraopes* in Blatchley (2). When brought into the laboratory, the beetles were placed in a cyanide jar. As soon as active movement had ceased each beetle was removed from the jar, the legs and wings were clipped, and the dorsal integument was cut, with a pair of fine scissors, along the mid line of the thorax and abdomen. Half the beetles were placed in 70% alcohol. The others were put in Bouin's fixative for about 12 hr. after which they were washed in repeated changes of 70% alcohol and then stored in 70% alcohol.

The gross structure of the stomodaeum and its relation to the mid-gut were studied in specimens preserved in alcohol. A beetle from the alcohol was pinned to a layer of wax in a watch glass and covered with water. The sides of the thorax and abdomen were pinned outward to disclose the digestive tract and it was found necessary to cut the head longitudinally and pin its halves outward in order to disclose the anterior portion of the crop.

Mounts of the lining of the stomodaeum were made from specimens preserved in alcohol. The digestive tract of a beetle was removed from the body cavity and the mid-gut was gently removed from its attachment to the oesophageal valve. One point of a fine pair of scissors was inserted into the valve and the stomodaeum was cut longitudinally along the length of its dorsal wall. The stomodaeum was then placed in a strong solution of potassium hydroxide for several hours until the muscles and other soft tissues had been dissolved. The hydroxide was washed out with water and the remaining sclerotized intima of the stomodaeum was washed with a series of alcohols of increasing strength. The alcohol was washed out with xylol and the intima was mounted in Canada balsam.

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[1] *Manuscript received April 2, 1949.*
Contribution from the Department of Zoology, McMaster University, Hamilton, Ont.

[2] *Assistant Professor of Zoology.*

Serial transverse sections of the stomodaeum were made from beetles that had been fixed in Bouin's fixative. They were sectioned at 10μ and were stained with haematoxylin and eosin.

Description

The stomodaeum of the beetle consists of a short, narrow oesophagus and an oval crop. The oesophagus (Fig. 2 – O) leads from the pharynx and is about 0.2 mm. wide and at its posterior end widens into the oval crop (C), which is about 2 mm. long. Most of the crop is enclosed within the head of the insect and only about one-quarter of its length extends through the foramen magnum of the head (Fig. 4 – C, F) into the thorax. The posterior end of the crop lies embedded in the anterior end of the mid-gut (Fig. 2 – MG). At this point the stomodaeum is sharply constricted and projects into the lumen of the mid-gut in the form of a rosettelike oesophageal valve with a narrow opening (Fig. 4 – V). The proventricular region of the stomodaeum is not distinguishable from the rest of the crop externally and consists merely of that portion at the posterior end of the crop that narrows toward the oesophageal valve.

The lining of the stomodaeum consists of a sclerotized intima (Fig. 1). In the crop it bears several low longitudinal ridges (LR). As these approach the constriction of the oesophageal valve (V) they anastomose to produce ridges that are fewer in number and finally, at the valve, they end in six poorly defined flaps (FL) that project into the mid-gut. The posterior half of the intima of the stomodaeum, including the longitudinal ridges and the oesophageal valve, is beset with a coating of small flattened spines (Fig. 3 – S). They are arranged in a regular, overlapping pattern, each spine being about 5μ long, broad at the base and tapering abruptly to a point that projects posteriorly in the stomodaeum.

In transverse section the longitudinal ridges of the anterior part of the crop are seen to project into the lumen (Fig. 5 – LR). They are covered with a layer of sclerotized intima (I), which is irregular in outline and about 10μ thick. Within the longitudinal ridges is a single layer of irregular epidermal

FIG. 1. *Sclerotized intima of posterior part of stomodaeum.*
FIG. 2. *Stomodaeum and anterior end of mid-gut.*
FIG. 3. *Spines of posterior part of stomodaeum.*
FIG. 4. *Posterior view of head showing stomodaeum (with mid-gut removed) projecting through foramen magnum.*
FIG. 5. *Transverse section of anterior portion of stomodaeum.*
FIG. 6. *Transverse section of posterior portion of stomodaeum.*
FIG. 7. *Transverse section of oesophageal valve and anterior end of mid-gut.*

C – crop; CM – circular muscle; EP – epidermis; EPi – inner epidermis of oesophageal valve; EPii – outer epidermis of oesophageal valve; F – foramen magnum of head; FL – flap of oesophageal valve; I – intima of stomodaeum; Ii – inner intima of oesophageal valve; Iii – outer intima of oesophageal valve; LM – longitudinal muscle; LR – longitudinal ridge; MG – mid-gut; O – oesophagus; S – spine of stomodaeum; V – oesophageal valve.

cells (EP). In some of the larger ridges are a few strands of longitudinal muscle (LM). The outer covering of the crop is a layer of striated circular muscle (CM) two or three strands thick. The posterior end of the crop is partly surrounded by a crescentic portion of the anterior end of the mid-gut (Fig. 6 – MG). The longitudinal ridges (LR) are fewer in number and larger than in the anterior part of the crop. The intima (I) presents a serrated

Fig.1.

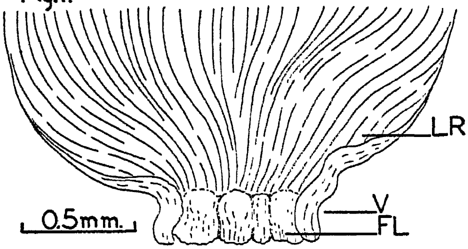


Fig.2.

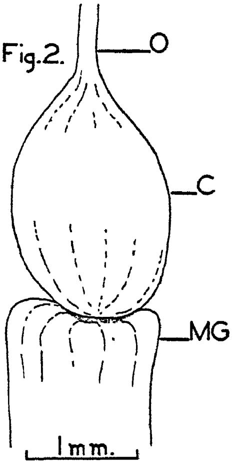


Fig.3.

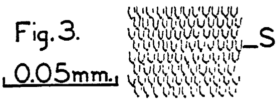


Fig.4.

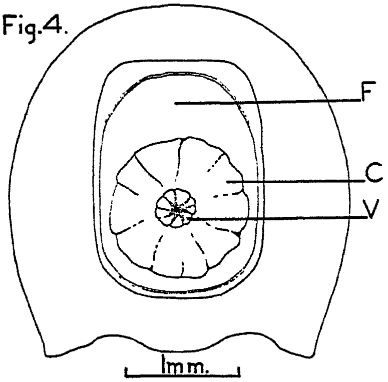


Fig.5.

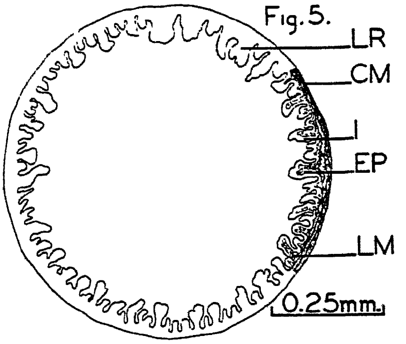


Fig.6

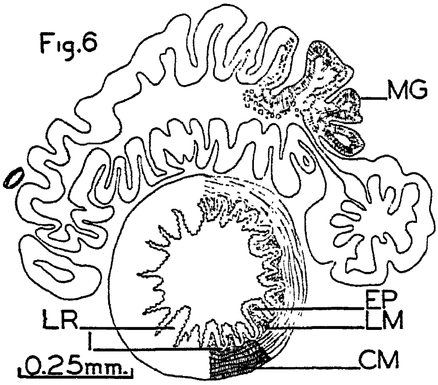
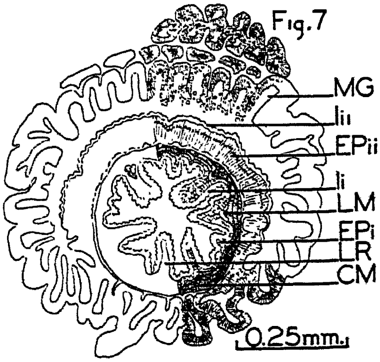


Fig.7



appearance owing to the presence of the small spines on its surface. The circular muscle (CM) is about twice as thick as in the anterior part of the crop and consists of four to six strands. The oesophageal valve lies within the anterior end of the mid-gut (Fig. 7 – MG). It has six large longitudinal ridges (LR) projecting into the lumen and a few smaller ridges between them. The intima (Ii) of the ridges is 10 to 20μ thick and shows the serrations caused by the spines on its surface. The intima (Iii) also appears on the outside of the oesophageal valve. The inner layer of the epidermis (EPi) consists of roughly cubical cells while the outer layer (EPii) consists of longer, columnar cells. The circular muscle (CM) is three or four strands thick.

Discussion

According to Snodgrass (5) the proventriculus in insects, in its simplest form, is "merely the narrowed posterior end of the stomodaeum which is more or less invaginated into the anterior end of the mesenteron to form the cardiac valve". The proventriculus of the milkweed beetle is clearly of this type being indistinguishable externally from the rest of the stomodaeum, and having only a coating of small spines to distinguish it internally from the anterior part of the stomodaeum. In the genera of Cerambycidae studied by Plateau (3) it is pointed out that there is no "gésier" or proventriculus and Thiel (6) reports that in the Polyphaga in general the proventriculus is poorly developed in contrast to the proventriculus of the Adephaga, which is a definite organ in the digestive tract. Balfour-Browne (1) reports that in some Cerambycidae there is "nothing more than a thin-walled expansible crop".

Some authors have attempted to correlate the structure of the proventriculus with the type of food eaten by insects, while others have attempted to correlate it with the manner of feeding. Ris (4) maintains that the proventriculus is not well developed in insects that feed on liquids, while it is well developed in those that chew solid foods. Plateau (3), however, points out that many insects that eat solid foods have no proventriculus. Ris says that insects that have well developed proventriculi devour their food and then cease feeding, whereupon the proventriculus rechews the food. Such insects may be called "intermittent feeders" and are usually active forms and possess a large crop. In inactive insects that feed continuously (e.g. lepidopterous larvae) there is not a well developed proventriculus and the crop is small. The stomodaeum of *Tetraopes tetraophthalmus* belongs in this latter category since it consists of a small crop and a poorly defined proventricular region.

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THE EFFECT OF GONADAL HORMONES ON LIVER NUCLEIC ACIDS IN THE IMMATURE PULLET[1]

By D. G. Chapman,[2] A. A. Hanson,[3] R. H. Common,[4] and W. A. Maw[5]

Abstract

The ribonucleic acid, desoxyribonucleic acid, and the ratio of ribonucleic acid to desoxyribonucleic acid in the liver of immature pullets treated with 4.5 mgm. testosterone propionate over a 12-day period were all increased when this treatment was supplemented by treatment with estrogen. The possible significance of these effects in relation to other effects of similar gonadal hormone treatments is discussed.

Introduction

Treatment of pigeons or immature pullets with estrogen increases the weight of the liver and the weight of the liver crude protein per kgm. live weight (3, 4, 5). The magnitude of these particular effects is not appreciably influenced by concurrent small doses of androgen, even though such androgen treatment is sufficient to enhance the effects of estrogen on the oviduct and to enable estrogen to increase the rate of calcium retention (6). Evidence as to an increase of liver weight and liver crude protein during the normal transition to laying activity is less clear-cut. However, high egg production in hens has been associated with heavier livers (8). It is highly probable that the increased endogenous estrogen production associated with the onset of laying tends to produce an increase of liver weight and of liver crude protein per kgm. live weight. Increases in liver weight and liver crude protein evoked by estrogen are not merely a consequence of the increased appetite (2, 15, 16) of estrogenized birds, for estrogen produces such effects in immature pullets when the food intake is maintained equal as between estrogenized and unestrogenized pullets.

The increases in serum phosphoprotein and phospholipid that normally accompany the onset of egg laying may reasonably be ascribed to endogenous estrogen activity. Estrogens are known to evoke similar changes in the sera of immature pullets and in cockerels (4, 5) and in male, female, and castrate pigeons (11). The sera of comparable untreated immature females and males

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contain little or no phosphoprotein. Since the increase of serum phosphoprotein takes place in the absence of the ovary, the phosphoprotein is most probably synthesized elsewhere than in the ovary. If the liver is involved, then the increase in liver crude protein under the influence of estrogen might be related to the appearance of phosphoprotein synthesizing activity as well as to an increase in synthesis of phospholipid.

There is evidence (7) that tissues engaged in active protein synthesis tend to have a relatively high ratio of ribonucleic acid phosphorus (RNAP) to desoxyribonucleic acid (DNAP). Novikoff and Potter (12) have related high RNAP : DNAP ratios in chick embryos to the periods of most rapid protein synthesis. If the effects of gonadal hormones on the livers of immature pullets include an increase of protein synthesizing activity, then such livers would be expected to show an increase in the ratio RNAP : DNAP. This change might be expected even when food intake is held constant so as to rule out the effects of increased appetite. Increase of protein intake might of itself affect the ratio RNAP : DNAP (9).

The present paper deals with experiments designed to test this theory. The experiments are an extension of previous experiments on the effects of gonadal hormones on immature pullets (4, 6).

In these previous experiments it was found that estrogen supplemented with a small testosterone propionate treatment produced a closer imitation of the normal puberal changes in the pullet than did estrogen alone (6). Such small testosterone propionate treatments by themselves did not appreciably affect the liver weight or liver crude protein as compared with those of untreated pullets. In view of these results, and since the number of pullets that could be properly handled was limited, the present experiment was limited to studying the effect, if any, of estrogen plus a small testosterone propionate treatment on liver nucleic acid phosphorus as compared with the effect of the testosterone propionate alone.

Experimental

Outline of Experiment

Two groups each of 12 immature Barred Plymouth Rock pullets were used. The birds in each group were taken from the same hatching and had been reared together under the same conditions.

The birds in each group were placed at random in individual laying battery cages arranged in three blocks of four cages each. The amounts of food eaten by the different birds in a group were kept as nearly equal as possible. Control of food intake in such experiments is of fundamental importance, since gonadal hormones stimulate appetite (2, 15, 16). Moreover, nutritional factors are known to influence the RNAP : DNAP ratio of liver tissues in the rat (9, 10).

In the first group the birds were fed on a commercial chick starter. In the second group the birds were fed on the following mixture:

Ground wheat	25 lb.
Ground corn	25 lb.
Ground barley	25 lb.
Wheat middlings	5 lb.
Soybean oil meal	15 lb.
Fish meal	2.5 lb.
Salt	1.0 lb.
Calcium carbonate	2.0 lb.
Fortified fish oil (2200 I.U. vitamin A per gm.)	0.25 lb.
Manganese sulphate	6 gm. per 100 lb. mixture

The hormonal treatments were given by intramuscular injection in six equal doses on alternate days. The total amounts of oily base injected were equalized. The birds were decapitated on the second morning after the last injection and allowed to bleed as freely and completely as possible. When bleeding had stopped the livers were rapidly removed and at once placed in tared screw-capped bottles in the ice chest at approximately −5° C. The livers were held in the ice chest until immediately before subsampling for analysis, and the analyses were all begun within a few hours of killing. It is essential to take all feasible measures to reduce nuclease activity in such work, since any appreciable nuclease action will introduce gross errors of observation.

Analytical Methods

The subsamples were dispersed in 10% trichloroacetic acid in a Waring Blendor. A little Hy-flo Super-Cel was added, and after further dispersion the suspended material was filtered off, washed successively with 1% hydrochloric acid and a little water, and then air-dried. Lipids were next removed by refluxing five times with Bloor's mixture (three volumes ethanol to one volume ethyl ether). The material was finally dried, weighed, and stored in the refrigerator pending analysis.

The RNAP and DNAP content of these residues was determined by Schneider's (14) method.

The difference between the total phosphorus of the residues and the total phosphorus extracted by hot 5% trichloroacetic acid was taken as a measure of the phosphoprotein phosphorus present.

Crude protein was determined by micro-Kjeldahl. Serum calcium was determined by the method of Halverson as described by Peters and Van Slyke (13). Dry matter was determined by drying to constant weight at 105° C. Phosphorus was determined by the method of Berenblum and Chain (1).

Experimental Results

The average results for all 24 pullets are set out in Table I and Table II.

TABLE I

EFFECTS OF TREATMENTS WITH GONADAL HORMONES ON OVARIES, OVIDUCTS, SERUM CALCIUM, AND LIVERS OF IMMATURE PULLETS (AVERAGES)

No. of pullets treated	6	6	6	6
Total dosage of testosterone propionate, mgm.	6 × 0.75	6 × 0.75	6 × 0.75	6 × 0.75
Total dosage of estradiol dipropionate, mgm.	6 × 0	6 × 1.0	6 × 2.0	6 × 4.0
Initial live weight, kgm.	1.01	0.97	1.05	1.08
Final live weight, kgm.	1.16	1.15	1.20	1.22
Ovary, gm.	0.30	0.28	0.26	0.25
Ovary, condition	Quiescent	Quiescent	Quiescent	Quiescent
Oviduct, gm.	0.27	16.8	18.9	21.2
Serum Ca, mgm./100 ml.	13.2	33.9	87.8	117.5
Liver weight, gm.	21.0	27.0	29.2	32.9
Liver, gm. per kgm. live weight	18.1	23.7	24.5	26.9
Liver crude protein, %	19.2	18.8	17.9	18.3
Liver crude protein, gm. per kgm. live weight	3.4	4.5	4.4	5.0

TABLE II

EFFECTS OF TREATMENTS WITH GONADAL HORMONES ON LIVER NUCLEIC ACID PHOSPHORUS OF IMMATURE PULLETS. AVERAGES FOR SAME BIRDS AS IN TABLE I

No. of pullets treated	6	6	6	6
Total dosage of testosterone propionate, mgm.	6 × 0.75	6 × 0.75	6 × 0.75	6 × 0.75
Total dosage of estradiol dipropionate, mgm.	6 × 0	6 × 1.0	6 × 2.0	6 × 4.0
RNAP, mgm. per 100 gm. fresh liver	55.6	57.6	61.8	59.6
Liver RNAP, mgm. per kgm. live weight	10.0	13.8	15.2	16.1
DNAP, mgm. per 100 gm. fresh liver	32.4	28.4	26.0	23.8
Liver DNAP, mgm. per kgm. live weight	5.83	6.73	6.35	6.40
$\frac{\text{Average RNAP, mgm. per 100 gm. fresh liver}}{\text{Average DNAP, mgm. per 100 gm. fresh liver}}$	1.72	2.03	2.38	2.50
$\frac{\text{Liver RNAP, mgm. per kgm. live weight}}{\text{Liver DNAP, mgm. per kgm. live weight}}$	1.72	2.05	2.40	2.52
RNAP, mgm. per 100 gm. liver crude protein	290	306	345	326
DNAP, mgm. per 100 gm. liver crude protein	169	151	145	130
Phosphoprotein P, mgm./100 gm. fresh liver	1.5	4.0	5.0	9.0

The data in Table I for ovary weights, oviduct weights, serum calcium, liver weights, and liver crude protein are in agreement with results previously reported (4, 5), and serve to define the state of the pullets.

The data for RNAP and for DNAP per kgm. live weight were analyzed statistically in order to measure the significance of the effects of estrogen. The possibility that initial live weights might have influenced these results was examined by covariance. The covariance test showed that differences in initial live weights did not account for the treatment effects under the given experimental conditions.

The data for RNAP and for DNAP per kgm. live weight were subjected to an analysis of variance. The main features of the analysis are presented in Table III.

TABLE III

ANALYSIS OF VARIANCE OF DATA FOR RNAP AND DNAP PER KGM. LIVE WEIGHT

Source of variance	D.f.	Mean squares	
		RNAP, mgm. per kgm. live weight	DNAP, mgm. per kgm. live weight
Treatments:	3		
Estrogen vs. no estrogen	1	112.001**	1.967**
Single vs. double and quadruple dose estrogen	1	13.813*	0.513
Double vs. quadruple dose estrogen	1	2.901	0.008
Groups (i.e. experiments)	1	114.407**	4.770**
Treatments × groups	3	9.905*	0.555
Blocks	4	12.132*	0.942**
Remainder	12	2.348	0.162

**Significant at 5% level.*
*** Significant at 1% level.*

Estrogen treatments increased the liver RNAP per kgm. live weight to a highly significant degree. The double and quadruple dosages of estrogen produced a significantly greater amount of liver RNAP than the single dosage. The quadruple dosage did not, however, produce a significantly greater amount of RNAP than the double dosage. It may be remarked that the difference between estrogenized pullets and those receiving no estrogen remains significant when compared with the mean square for treatments × groups.

The estrogen treatments also evoked a highly significant increase in liver DNAP per kgm. live weight, but the differences between birds on different levels of estrogen were not significant in this case. Even in the case where estrogen increased the amount of liver DNAP per kgm. live weight, therefore, a relatively greater increase was brought about in liver RNAP.

Discussion

The data now reported prove that when estrogen treatments are superimposed upon androgen treatments sufficient to evoke puberal comb and wattle changes in sexually immature pullets, then under the given conditions the estrogen (*a*) increases total liver DNAP, but to a limited extent; (*b*) increases liver RNAP; and (*c*) increases the ratio RNAP : DNAP in the liver.

It is already known (4, 5) that the increase of liver crude protein per kgm. live weight evoked by estrogen is not seriously modified by concurrent administration of amounts of testosterone propionate that are adequate to produce puberal comb and wattle changes and to enhance the effects of estrogen on hypertrophy of the oviduct. When the present results (*vide* Tables I and II) are examined in the light of this observation, then it would seem that estrogen changes the liver protein qualitatively as well as quantitatively. The total liver DNAP is increased by estrogen, but the amount per 100 gm. liver crude protein falls. The total liver RNAP and the amount per 100 gm. liver crude protein both increase. The amount of liver RNAP + DNAP per 100 gm. liver crude protein displays a remarkable tendency to constancy.

Before proceeding to a tentative interpretation of these results, however, it must be recalled that the livers were not perfused and that reliance was placed on free bleeding after decapitation to free the livers from appreciable remaining amounts of blood. (Perfusion will ensure removal of blood, but it was considered more desirable to avoid the probability of extensive nuclease action during prolonged perfusion than to remove the last traces of blood.) Estrogen evokes marked changes in blood composition. One cannot, therefore, completely dismiss the possibility that the differences in RNAP and DNAP may be a reflection of differences in amounts of blood remaining in the livers. Conclusive evidence on this point is still lacking, but the liver material seemed remarkably free from blood during preparation.

Conclusions

Pending the detailed investigation of residual blood and of nuclease action in the livers of such experimental pullets, therefore, the following tentative conclusions may be drawn from these experiments:

(1) Treatment of the immature pullet with estrogen combined with androgen increased the amount of liver crude protein per kgm. live weight, in confirmation of previous observations. These increases have now been found also to modify the relative proportions of ribonucleoprotein and desoxyribonucleoprotein in the liver. The effects are independent of any stimulatory effect of estrogen on appetite and protein intake.

(2) The modifications were such as would be expected from an increase in protein synthesizing function or the acquisition of new protein synthesizing functions. Increasing doses of estrogen increased the total amounts of liver RNAP and DNAP and also increased the ratio RNAP : DNAP in the liver. The total amount of RNAP plus DNAP per 100 gm. liver protein remained relatively constant.

(3) It may be suggested tentatively that endogenous gonadal hormone activity will tend to evoke similar changes in the liver when reproductive activity begins normally.

Acknowledgments

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EXCHANGE OF MATERIALS IN A LAKE AS STUDIED BY THE ADDITION OF RADIOACTIVE PHOSPHORUS[1]

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Abstract

On July 1, 1948, 100 millicuries of radioactive phosphorus, P^{32}, was added to the surface of an acid bog lake of area 0.3 hectares (0.8 ac.) and depth 6 m., situated near Halifax, Nova Scotia. The lake had a well developed zone of cold, stagnant water at the bottom with a thermocline above. Within a few hours the P^{32} was taken up strongly by the sphagnum (which made up most of the lake margin), by the floating plankton, and by sponges. Within a few days it had reached the bodies of fishes (*Fundulus* and *Notemigonus*), and within a fortnight had accumulated in their skeletons and viscera. No appreciable uptake of P^{32} by such bottom rooted forms as the cranberry (*Vaccinium*) and leather leaf (*Chamaedaphne*) was observed for more than two weeks. Penetration of added phosphorus into the lake depths was very slow, and even at the end of eight weeks the material was only doubtfully detected at the bottom. There was no evidence of active uptake by bottom mud. The ratios of maximum count per wet weight of organism to maximum count for the same quantity of lake water were as follows:

Zooplankton	40,000
Fundulus	13,000
Sponge	4500
Sphagnum	400
Vaccinium	300
Blue-green alga	300
Chamaedaphne	100
Nuphar	80

One morning during the summer of 1948, 100 millicuries (mc.) of radioactive phosphorus, P^{32}, was added to a small lake in the vicinity of Halifax, Nova Scotia. The purpose of this paper is to describe the utilization of the phosphorus by plants and animals and its penetration into the water. The experiment was essentially similar in technique to familiar tagging experiments in which selected animals are marked in some way, then released. When the animals are taken again, the migration is evident.

The Lake

The lake in question is situated 12 mi. from Halifax, 100 yd. from the St. Margaret's Bay Road. It occupies an area of about 0.3 hectares (0.8 ac.), and contains some 16,000 cu. m. of water. The lake is fed by an inlet supplying approximately 4 cu. m. per hour during the summer season, while the outlet was observed to carry away some 5.5 cu. m. per hour, the difference being presumably due to seepage.

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Botanical characteristics of the lake are indicated in Fig. 1, which gives a typical picture of an acid bog lake, filling in by encroachment from without. The bordering region is a heath association, largely *Chamaedaphne* and *Myrica Gale*, that gradually merges into the sphagnum margin of the open water. Ninety per cent of the margin consists of a wide mat of sphagnum, which, being covered with heath growth, produces a springy mat that is gradually filling in the lake. The fundamental shape of an acid bog lake is that of the bulb of a thistle tube, the area being greater some distance below the surface than at the surface.

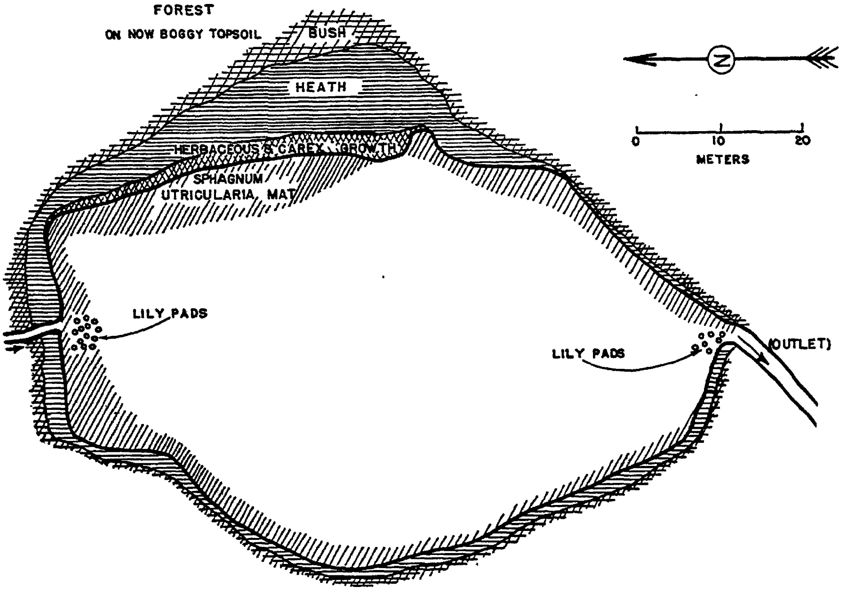


FIG. 1. *Botanical characteristics of the lake used for experiments.*

Although the lake is very small and its description seems to answer to that of a pond, its depth varies from 3 m. near shore to 7 m. at its deepest part. There was a thermocline and hypolimnion, which are shown in Fig. 2. The thermocline extended down for some three meters, below which the temperature was only some 5° C. throughout the summer.

Fig. 3 shows the oxygen characteristics of the lake. At no part was there saturation with oxygen and a marked gradient existed so that below three meters oxygen was very scarce, and below five meters undetectable. A peak in the oxygen curve at a depth just below one meter is not easy to explain, but has been observed in a number of lakes in this region.

The oxygen and temperature characteristics of the lake are such that the depths had too little oxygen for trout, while the surface waters in summer might be uncomfortably warm. At any rate no trout were observed in the lake during the experiment. The only two native fishes taken were *Fundulus diaphanus* and *Notemigonus crysoleucas*. As these fishes were in scarce supply and as it was desired to obtain samples at intervals, some four thousand *Fundulus* were seined from neighboring lakes and introduced into the experimental area a few days before the addition of P^{32}.

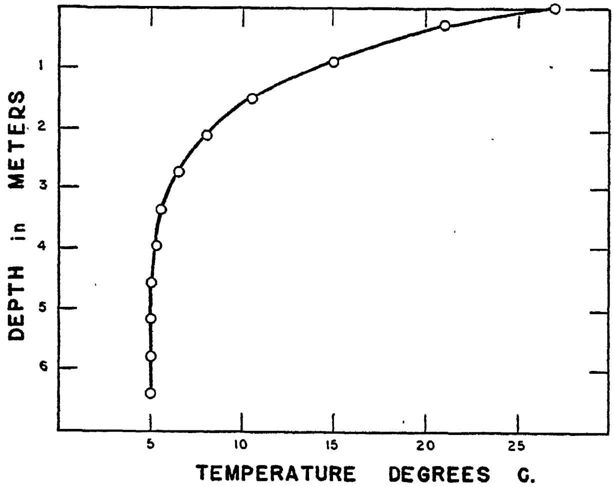


FIG. 2. *Variation in lake temperature with depth. Observations were made in the morning and show a thermocline and hypolimnion. Doubtless a series of observations made in late afternoon on a sunny day would show an epilimnion as well.*

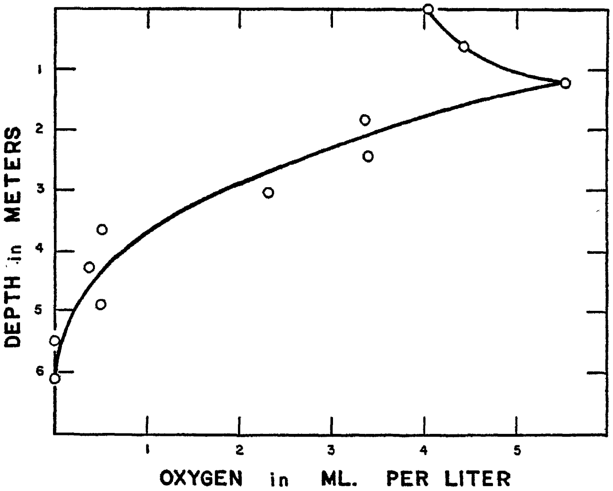


FIG. 3. *Variation in dissolved oxygen of the lake with depth.*

Methods

A quantity of P^{32} having a stated activity of 100 mc. on June 25, 1948, at 2.00 p.m. was obtained from the laboratories of the National Research Council. At the time of its introduction into the lake the presumed activity was about 76 mc. This material, dispersed in 27 gm. of potassium dihydrogen phosphate was dissolved in water in a glass container of about 25 liters capacity. The method of solution, in order that the greatest precaution be taken, consisted of lowering the vial of P^{32} on a cord into the larger vessel of water, the contents of the vial then being dissolved by pumping a continuous stream of water into it with a syringe bulb and tubing. The large container of active phosphate was placed on a platform on a rubber boat and pushed back and forth across the entire surface of the lake by another boat, from which was operated a syringe bulb that pumped air into the jar and started a siphon by means of which active liquid was delivered into the water. Propulsion provided by an outboard motor served to mix the surface water thoroughly. The method allowed the active material to be well isolated, the vessel being situated about 10 ft. from the man controlling the siphon. Deposition required about one hour and was completed on July 1 at 7.00 a.m., which is called "zero hour". The sun was shining, and the water dead calm. The technique of deposition was tried out by a dummy run on the day previous to the actual experiment.

Total phosphorus in the lake was measured before the experiment, by the method of Robinson, using a Lumetron photoelectric colorimeter. Surface values averaged 25 parts per billion and those in deeper water 15 p.p.b. Thus the lake of 16,000 cu. m. would contain some 320 gm. of phosphorus, and 27 gm. of potassium dihydrogen phosphate, containing 6 gm. of phosphorus, would increase the natural store by 2%. If we take the inorganic phosphorus to be about 1/10th of the organic in this type of lake, it might be that the enhancement of inorganic phosphorus amounted to some 20%. However, the ratio of organic to inorganic phosphorus is not accurately known, the latter being insufficiently concentrated for accurate estimation, as is the case in most Halifax County lakes.

Samples of lake water were collected at various depths using a Foerst Water Bottle. Mud samples were taken with an Ekman dredge. Lake temperatures were measured with a Leeds and Northrup "Thermohm" Electric Resistance Thermometer. Oxygen estimations were made by the Winkler method. Plankton samples were collected in a conventional silk net.

The methods for determining the quantity of P^{32} in the water consisted of evaporating the sample (100 ml. in initial tests) to about 3 ml. using gas burners and fans under a fume chamber. Evaporation to dryness was carried out in aluminum counting trays on hot plates. As the activity in the water decreased, larger samples were evaporated, 900 ml. being the largest amount used. The trays were then placed in a lead castle and counted with Geiger tube and scaler. Evaporation was, for several reasons, more satisfactory than precipitation with sulphate–molybdate, which was also tried. Precipitation and evaporation methods checked satisfactorily.

Fish were prepared for the phosphorus counts by ashing in a perchloric acid and nitric acid mixture. The phosphorus was precipitated with sulphate–molybdate reagent, filtered, and counted on the filter papers.

The plankton samples were collected in a Büchner funnel on discs of filter paper cut to the correct size to fit a counting tray. The dried material was then placed directly in the counter.

Sphagnum was first dried, then ground with mortar and pestle, and subsequently spread evenly over tared counting trays. After weighing, the position of the sphagnum was fixed with label glaze.

Sponges, and higher plants, were treated in essentially the same way as sphagnum.

Four counters and scalers were used, being brought into relation with each other by counts on a standard phosphorus sample. For a number of reasons variations occur in all counters from day to day. Allowance was made for these by daily counts of standard uranium samples. Conditions of geometry that prevailed at the time of standardization of the four counters were maintained throughout the experiment. The sample thickness in no case warranted the application of a correction for self absorption.

In all the statements of counts and in the illustrations in this paper, corrections for decay have been applied in such a way as to bring the P^{32} to the value that it had at the time it was standardized before shipment. No additional standardization was carried out, it being presumed that the quantity of P^{32} was as accurately known as other major variables in the experiment, such as the lake volume or the amount of natural phosphorus in the lake.

The method for converting counts of the samples into counts per minute per 100 gm. of material as used on the graphs was as follows:—Consider the point on Fig. 7 for 28 hr. after zero hour. The plankton sample was taken from the lake at 11 a.m. on July 2 and counted at 8 p.m. on July 5 with Geiger–Müller tube No. 3. The dry weight of such a sample was 0·0698 gm. The ratio of dry weight to wet weight was 4.5. The counts per minute on the sample were 4585 and the background was 11 counts per minute. The uranium standard gave 9404 as compared to 9660, which had been taken as a standard count on standard day. At 8 a.m., July 5, only 60% of the original activity was left, which meant that the count must be multiplied by 100/60 in order to bring the count up. The factor relating counter No. 3 to counter No. 1 (standard counter) was 2.384. The corrections thus applied give the figure used on graph from the following expression:

$$\text{Counts per minute} = (\overset{(a)}{4585} - \overset{(b)}{11}) \times \overset{(c)}{\frac{9660}{9404}} \times \overset{(d)}{\frac{100}{60}} \times \overset{(e)}{2.384} \times \overset{(f)}{\frac{100}{0.0698 \times 4.5}} = \overset{(g)}{5.95} \times 10^{6}$$

(*a*) = original count on counter
(*b*) = background
(*c*) = ratio correcting for fluctuation in counter
(*d*) = correction for decay
(*e*) = factor relating counter No. 3 to standard counter
(*f*) = conversion to 100 gm. wet weight
(*g*) = c/m as seen on Fig. 7.

Radioautographs were made by pressing the dried or frozen samples against a piece of high-speed dental X-ray film, first placing a piece of waterproof plastic between the sample and the emulsion. The fish were frozen and sagitally sectioned leaving, however, the vertebral column intact. Such specimens were kept frozen during the whole exposure time. Exposure times were empirical and, in general, lasted from 1 to 15 days, depending on the concentration of P^{32}.

Penetration of P^{32} into the Depths

Analyses of mud from the lake bottom were made from time to time, but no appreciable concentration of P^{32} was observed. Water samples were taken at a station over the deepest part of the lake, the counts being shown in Fig. 4.

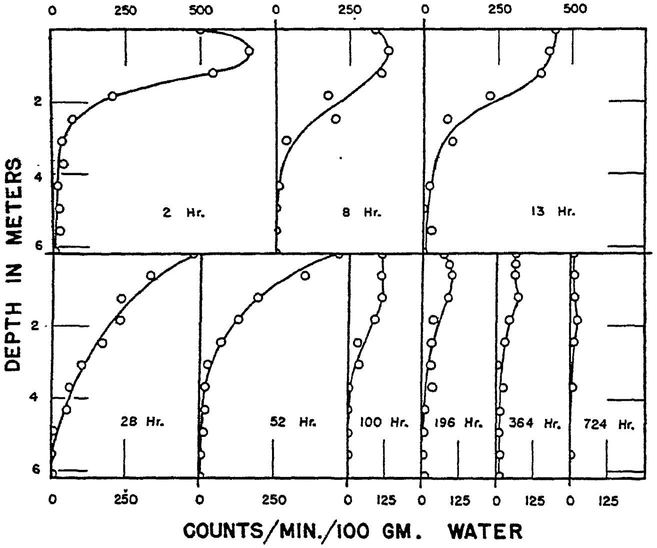


FIG. 4. *Counts per minute of P^{32} at different depths and at increasing intervals after the time of addition. Each point on the curve represents one determination. All counts were made at one station over the deepest part of the lake.*

From very early in the series some counts of phosphorus were found in the deep waters of the lake, although these were so small compared with surface counts that they might be due to water carried down with the depth sampling bottle. The first evidence of penetration below the thermocline was observed at 28 hr. At no time up to the end of observations was the P^{32}, added at the surface, clearly detected in the deepest water.

One further feature of Fig. 4 deserves mention, namely, a peak below the surface that is first noticed at 196 hours. This peak is believed to be caused by a runoff of surface water in the lake. It might be expected that such a peak would gradually move down into the depths of the lake as warmer water was removed from the surface, and this is what seems to be occurring in the two final readings.

There are two reasons why added phosphorus could appear in the depths of the lake, one of them being turbulence, and the other being due to accumulation of P^{32} in the bodies of plankton organisms at the surface. Plankton exhibits diurnal vertical migratory behavior and could therefore take phosphorus to the bottom. Dead plankton organisms might also sink to the bottom giving up their phosphorus in the deeper layers of water.

Fig. 5 is an attempt to follow the fate of the added phosphorus as it disappeared from the lake. The horizontal line at the top is the theoretical value for counts, of the initial addition of 100 mc. of P^{32}. The curve (or lower curve) gives the total disintegrations in the lake. The upper curve (which has the lower curve as its base line) shows the cumulative discharge of the outlet stream. Thus the upper block of the diagram represents the uptake by organisms, etc., within the lake. It is apparent that four-fifths of the added phosphorus was utilized, largely by organisms, within three weeks.

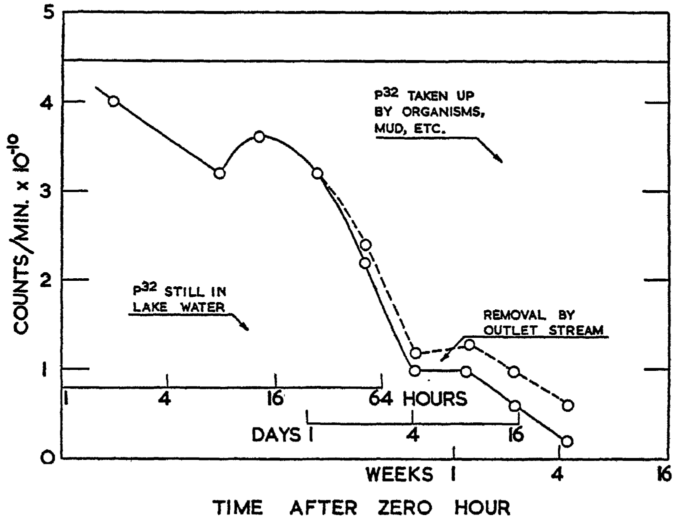


FIG. 5. *Fate of the phosphorus added to the lake. Horizontal line at the top is the theoretical value for counts of the initial addition of 100 mc. of* P^{32}. *The points on the curve, or lower curve (solid line) are obtained from the data plotted in Fig. 4. Take for instance, the two hour curve of Fig. 4, and draw horizontal lines through each point, to divide the lake into a number of layers, each 0.61 m. (2 ft.) thick. For each layer take as the* P^{32} *concentration, the average of the two points above and below it. Now from a contour map of the lake (not published) the volume in each layer is established. The total counts in each layer are:*

$$\frac{\textit{Average of two points in Fig. 4} \times \textit{total ml. water in layer of lake}}{100}$$

Summing up the values for all 10 layers one obtains the total number of counts in the lake at two hours, which is set down as the initial point in Fig. 5. It might be noted that readings for the deeper layers were often negligible, because P^{32} *was scarce and the contours closing in. If the lake has overhanging margins as suggested above, a constant volume of water under the margins would be overlooked in calculations. No allowance has been made for this.*

The upper curve (dotted line), which has the lower curve as its base, shows the cumulative discharge of P^{32} *by the outlet stream.*

The rapid disappearance of the P^{32} is rather disturbing to anyone thinking of adding fertilizer to natural waters as a fish cultural procedure. In the present case the increase in the phosphorus content of the water was only 2%, and it might be thought that if a great deal more had been added, there would have been enough to satisfy the sphagnum and fish and still leave the lake with a high phosphorus value for some time. General experience with added nutrients does not, however, support such an assumption. We have, for example, added massive amounts of phosphorus, in the form of basic slag, to acid bog lakes in the vicinity of the one here under discussion. Basic slag was selected because the phosphorus in it is liberated rather slowly, and it was hoped that a continuing supply would be given off. In so far as the hope was realized, the effect would be to hold up the phosphorus level of the lakes. Some results are given in Table I, from which it is apparent that neither the amount of phosphorus initially present in the lake, nor the amount added, stands in any clear relation to the rate of removal.

The lakes mentioned in Table I are shallow, of 5 to 10 ac. in area, and with a negligible runoff in summer. In all cases the theoretical amount of added phosphorus that could go in to solution was 450 mgm. per cu. m. This value is based on the immediate solubility of the phosphorus in basic slag in water of pH 5.5 and, as the table shows, was not reached in any case.

TABLE I

DISAPPEARANCE OF PHOSPHORUS WHEN ADDED TO ACID BOG LAKES. ANALYTICAL VALUES ARE IN MGM. PER CU. M. OF SURFACE WATER

Lake	Phosphorus in lake at start	Maximum observed, one or two days after addition	Phosphorus present after one month	Percentage of added phosphorus that disappeared in one month
South Weaver (1st addition)	15	75	45	50
South Weaver (2nd addition)	45	230	45	100
Holland (1st addition)	70	230	150	50
Holland (2nd addition)	150	475	230	75
This paper (P^{32})	20	—	—	90

Uptake by Sphagnum

Estimations at three stations on the margin of the lake were made from time to time, of P^{32} in sphagnum at the surface, and one-half meter below the surface, the results being shown in Fig. 6. It will be noticed that the base line of Fig. 6 has been drawn logarithmically, and the same is true of the curves to follow. Observations of sphagnum and other materials were made at logarithmically spaced time intervals so that the distances between the operations shown on Fig. 6 and other figures are approximately equal.

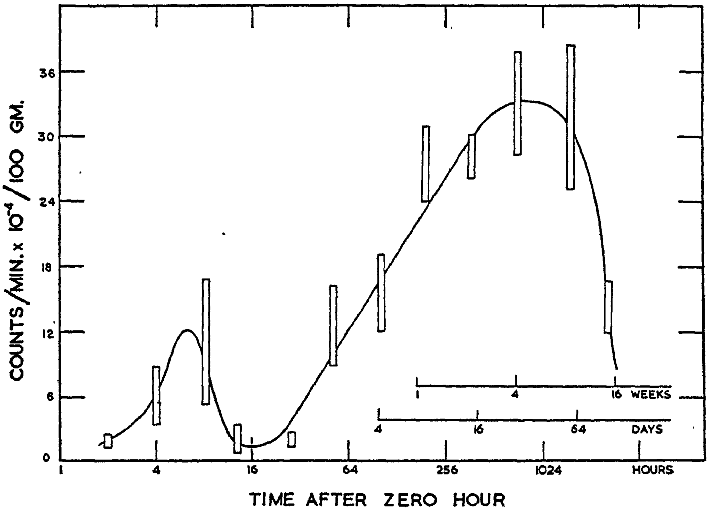


FIG. 6. *Variation in P^{32} counts in sphagnum from the lake. Observations were made at three stations on the lake margin, at each of which one sample of sphagnum was taken at the surface and one 0.5 m. down. Results of the six observations were averaged and the standard error calculated. Each vertical line on the figure shows the limits of the standard error of a mean.*

The vertical lines on Fig. 6 show the limits of the standard errors of the means, as will be clear from an example. Take the readings at approximately four days (100 hr.).

Marginal station	Depth	Counts per minute per 100 gm. sphagnum × 10^{-4}
A	Surface	10.3
A	0.5 m.	9.6
B	Surface	13.0
B	0.5 m.	7.0
C	Surface	26.0
C	0.5 m.	28.0

The average count was 15.7 and the standard error 3.7. Hence the limits were 19.4 and 12.0, between which the line has been drawn on Fig. 6.

The curve of Fig. 6 appears to exhibit two peaks, the validity of the first of which must be considered. It will be noticed that the first and fourth observations were low and the second and third were high. The difference between the means of the first and second observations is scarcely significant, and the same is true of the third and fourth. However, if we combine the second and third on the one hand and the first and fourth on the other, the difference becomes significant. We have therefore placed an initial peak in the curve.

It is interesting to note that the first peak in the sphagnum curve corresponds in time to a trough in the water curve of Fig. 5, and the subsequent trough in the sphagnum curve corresponds to a peak in Fig. 5. If it could be assured that a fall in sphagnum P^{32} caused a measurable rise of the lake P^{32}, it would be possible to determine the mass of participating vegetation. Further investigations under controlled conditions are in progress along these lines.

A glance at Fig. 6 shows that the uptake by sphagnum exhibited a second peak at about three weeks. It appears reasonable to suggest that sphagnum possesses two mechanisms of phosphorus uptake, the first of which might be due to physical processes unconnected with active cell metabolism. The second peak might be due to the incorporation of phosphorus into the protoplasm in some permanent way.

The decline after the second peak could be attributed to two causes. First, a loss of phosphorus could occur from the sphagnum back into the water. Second, it would be expected that the initial mass of cells containing P^{32} would be increased by the addition of new cells with diminishing quantities of P^{32}. This would have the effect of diluting the sample taken for analysis.

No significant difference was observed between the surface counts and those taken 0.5 m. down.

Plankton

Samples of plankton were collected from time to time, the analysis of results being shown in Fig. 7. No attempt was made to separate plankton into species or groups, but this, as a matter of fact, would not have improved the results because the plankton as collected turned out to be practically a pure culture of the copepod, *Diaptomus*. Fig. 7 does not exhibit a preliminary

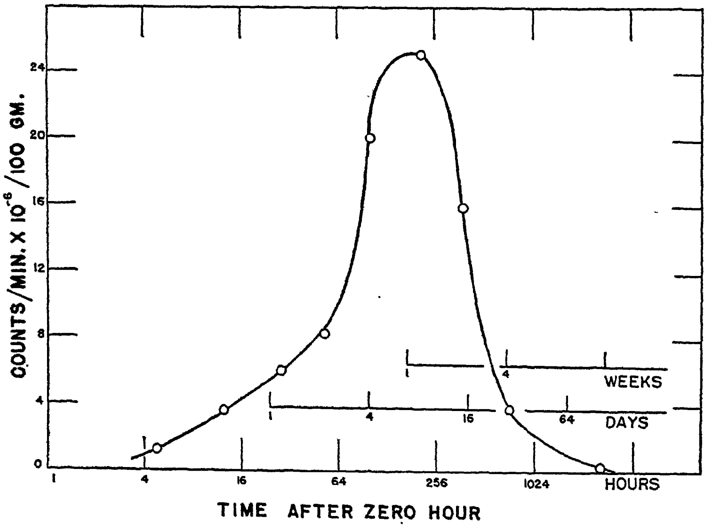


FIG. 7. *Uptake of P^{32} by zooplankton, which consisted almost wholly of Diaptomus.*

peak like that shown by sphagnum, but rises gradually to a single maximum at about 200 hr., after which the curve declines steadily. It should be noted that the situation as regards plankton is quite different from that of sphagnum, the former being composed of animals whose lives are of short duration so that the counts shown in Fig. 7 do not represent the conditions in a single generation of forms that live through the summer, but in several generations. The decline in Fig. 7 reflects merely the fact that there was less P^{32} in the water to be picked up by new generations of plankton at the time the later observations were made.

Fish

The curve describing fish, which covers observations on both *Fundulus* and *Notemigonus*, is given as Fig. 8. It will be noticed that no P^{32} could be found in the fish until some 50 hr. after the beginning of the experiment. Such a late beginning of deposition in fish contrasts strikingly with the early uptake observed in the plankton and suggests that a different mechanism may be operating in the two cases. In the case of the plankton it is easy to imagine that inorganic phosphate is taken up directly, while in the fish the time lag would suggest that the P^{32} must first be taken up by the plankton organisms and incorporated into their tissues. The fish would then receive phosphorus by eating the plankton.

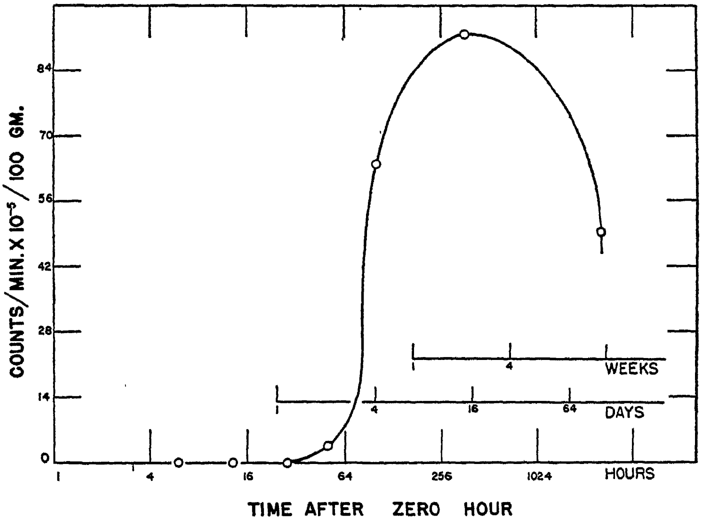


FIG. 8. *Uptake of radioactive phosphorus by two fishes, Fundulus and Notemigonus. Each point from analysis of one specimen.*

Obviously there is a considerable quantity of water swallowed by the fish. It will also be remembered that there is a continuous stream of water, going in through the gills by osmosis, which is eliminated by the kidneys. Despite this, however, there is apparently no selective storage of phosphorus in the fish tissues directly from the water.

The curve for fish exhibits a decline, with the peak at approximately the same place as for sphagnum, and the causes of the decline may be the same as given for sphagnum.

Other Organisms

As contrasted with the results already described, P^{32} was not observed in the leaves of marginal rooted organisms until a fortnight after the experiment began. Fig. 9 shows the counts on leaves of cranberry and leather leaf, both making up heath at the lake margin and collected by reaching out of a boat. The delayed appearance suggests that P^{32} reached the leaves via the roots. It may be presumed that P^{32} was first taken up by the roots and later reached the leaves. The time required for appearance in the leaves may be made up of (*a*) time to reach the roots, perhaps considerable; (*b*) time to penetrate the roots, perhaps very short; (*c*) time for translocation to the leaves. The difference in shape between the two curves of Fig. 9 may be related to the fact that the time of flowering and fruiting for *Chamaedaphne* was just over, while that for the cranberry was just starting.

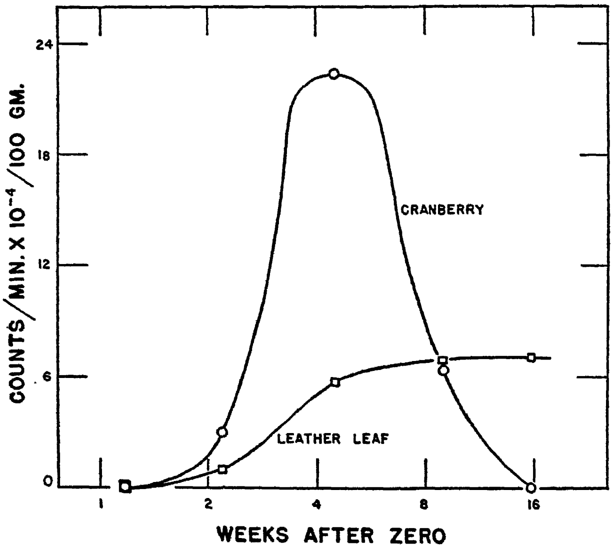


FIG. 9. *Concentration of P^{32} in the leaves of two higher plants at intervals after deposition. Both the leather leaf (Chamaedaphne) and the cranberry (Vaccinium) were rooted in the marginal bog.*

A further observation, which may be mentioned at this point, was made on the growing tips of *Hypericum*, the St.-John's-wort, which was also growing at the lake margin. Forty-eight hours after zero, the count per 100 gm. was 4×10^5, a somewhat higher value (two- to tenfold) than was ever recorded for the leaves of higher plants.

Fig. 10 illustrates the uptake of P^{32} by two additional organisms, yellow water lily leaves (*Nuphar*), and sponge. In these cases our observations began late (four days) so that the initial time of uptake cannot be precisely stated. The general structure of a sponge would lead one to expect an early uptake, while, by contrast, the water lily leaves would presumably have to obtain P^{32} via the roots, and hence exhibit some delay. The peak on the sponge curve might have the same cause as given above for sphagnum. It would be premature to attempt an interpretation of the water lily variations.

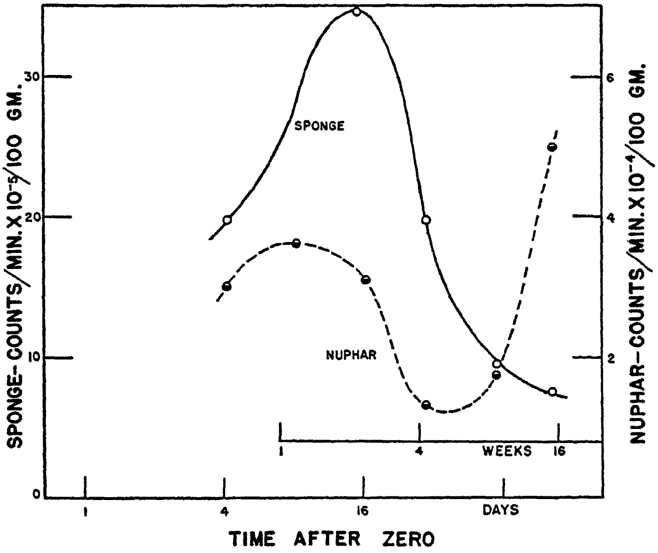


FIG. 10. *Amount of added phosphorus present in the sponge (Spongilla) and yellow water lily (Nuphar) at various times.*

Four counts on the alga *Batrachospermum* were also made between nine days and eight weeks from zero time. No trend was discernible, the average value being about 6 × 10^5 counts per 100 gm. By the time the first count was made the maximum value was already established. It is reasonable to think that such a form would take phosphorus up quickly, but proof is not at hand.

Comparative Uptake

It is of interest to measure the intensity of phosphorus assimilation by the various plants and animals in the lake. There are two ways of making such a calculation as shown in Table II.

First, we may give the absolute uptake, which is the ratio of the maximum observed value for an organism (peaks of curves) compared to the maximum value for the same weight of water (Fig. 4, subsurface at two hours). It is seen that animals take up more than plants; higher animals more than lower animals; and lower plants more than the higher plants. As regards the last statement, however, it must be remembered that the measurements on whole algae and on all exposed parts of sphagnum are not strictly comparable to the leaf counts made on higher plants. It was noted above that the growing tips of a higher plant gave greater counts than leaves.

TABLE II

COMPARATIVE UPTAKE OF P^{32}

Common name	Organism	Absolute uptake $\times 10^{-2}$. Ratio of maximum counts per 100 gm. organism to maximum counts per 100 gm. water	Relative uptake $\times 10^{-7}$. Maximum counts per min. per gm. total phosphorus in organism
Alga	*Batrachospermum*	10	660
Sphagnum	—	4	10
Cranberry	*Vaccinium Oxycoccus*	3	3
Leather leaf	*Chamaedaphne*	1	1
Yellow water lily	*Nuphar*	1	2
Sponge	*Spongilla*	45	500
Zooplankton	*Diaptomus*	400	13
Fish	*Fundulus* and *Notemigonus*	130	2

A second calculation gives the relative uptake, by answering the question: how much P^{32} can be taken up in proportion to the total phosphorus content of the organism? Fish will serve as an example of the method of calculation. The peak of the curve in Fig. 8 is observed to have a value of 91 × 10^5 counts. By conventional analysis fish were found to contain 0.56% of total phosphorus.

$$\text{Thus } \frac{91 \times 10^5}{0.56} = 1.62 \times 10^7 ,$$

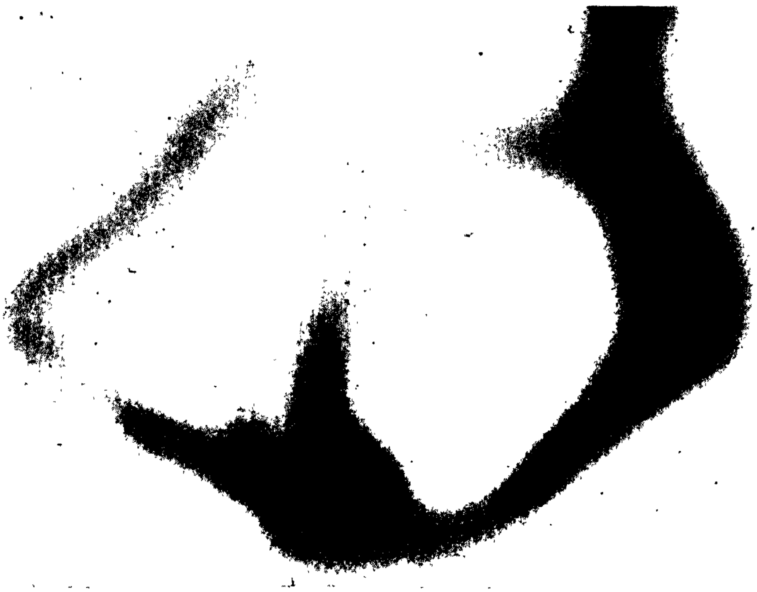
which has been set down as the nearest whole number, 2, in Col. 4 of Table II. The ratios in Col. 4 provide a comparison of the rate of turnover of phosphorus by each plant or animal. Maximal values are observed for alga and sponge, the simplest forms dealt with. Next, but greatly lower, come sphagnum and *Diaptomus.* Fish and higher plants made up a still more sluggish group. The high value for *Diaptomus* as compared to fish is, perhaps, related to (*a*) the short life of a generation of plankton organisms, making a rapid turnover of material necessary, and (*b*) the mass of inorganic phosphate in a fish skeleton, which is renewed fairly slowly (see further on).

In both columns of the table the sphagnum ratios are below some of the others. Nevertheless, sphagnum is believed to be the main remover of P^{32} in this lake, because of its great abundance.

Radioautographs

Radioautographs of a number of organisms were made from time to time with a view to determining the site of deposition of P^{32}. Fig. 11 is a picture of a sponge, *Spongilla,* taken from the lake four days after the beginning of the experiment, exposure time being 21 hr. A general darkening of the whole tissue is evident.

Fig. 12 is a frog that had been in the lake for 40 days, and was exposed to the film for 15 days. The phosphorus has become deposited in the skeleton and is quite clearly shown in the jaws and the limbs. The variations of



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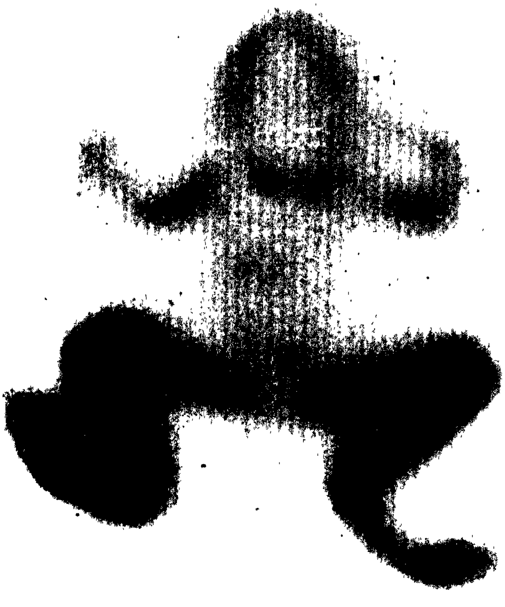

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FIG. 11. *Radioautograph of a sponge taken from the lake four days after addition of radioactive phosphorus. Exposure, 21 hr.*

FIG. 12. *Radioautograph of a frog taken from the lake 40 days after addition of P^{32}. A concentration of material in the skeleton is evident. Exposure, 15 days.*

PLATE II

13

14

FIG. 13. *Radioautograph of Fundulus taken from the lake nine days after the experiment started. The phosphorus appears to be distributed over the whole body, with some concentration in the viscera. A dark spot might be the liver. Exposed six days.*

FIG. 14. *Distribution of* P^{32} *in Notemigonus removed 16 days from the start. Concentration of the new phosphorus in the axial skeleton and at the bases of the fins is evident. Exposed 24 hr.*

intensity of darkening between fore and hind limbs do not indicate differences in phosphorus concentration, but the proximity of the parts to the photographic film.

Fig. 13 is a specimen of *Fundulus* taken seven days after the beginning of the experiment and exposed for six days. There is evident a general darkening over the whole body with some tendency for a concentration of P^{32} in the region of the gut. Possibly a heavily pigmented spot in the anterior gut region might represent the liver.

Fig. 14 is a specimen of *Notemigonus* taken 15 days from the start, and exposed for 24 hr. A considerable change is noticed from the preceding picture, for by this time the phosphorus had begun to show a marked concentration in the skeleton including both the vertebral column and the bases of the fins and tail.

Conclusions

1. P^{32} uptake by plants and zooplankton is a matter of minutes or hours, not days or weeks.
2. Sphagnum appears to have two mechanisms of phosphorus uptake.
3. Zooplankton can apparently either take up inorganic phosphorus directly, or feed with great rapidity on microorganisms that have absorbed the added phosphorus at once.
4. Fish evidently cannot take up inorganic phosphorus directly, but presumably obtain it by feeding on plankton.
5. Phosphorus taken up by fish is initially dispersed through the body generally, with some concentration in the gut tract area. Later it is segregated into the skeleton, both axial and appendicular.
6. Living organisms can concentrate added phosphorus to a level up to 40,000 times that of the water.
7. Estimations of soluble phosphorus do not appear to provide a reliable guide to the level of fertility, at least in this type of lake. Added phosphorus is quite rapidly removed. We may picture the phosphorus level at equilibrium, as determined by the tendency of plants (in proportion to their abundance) to pull phosphorus out of the water, balanced by replacement from decaying plants and by the inlet. Thus the same equilibrium level for phosphorus might be reached under a variety of conditions. There may, however, very well be a quantitative relation between the rate of removal of added phosphorus and plant abundance.
8. Fertilization of acid bog lakes with a view to fish production, appears to be relatively unprofitable, for the fertilizer will quickly be incorporated into the sphagnum. Most of the phosphorus in sphagnum goes to form peat although some may be later returned to the lake water.

Acknowledgments

From the standpoint of equipment, this work was made possible first by the fact that the National Research Council placed in the Chemistry Department of Dalhousie University the counters and scalers necessary to make estimations of P^{32} and second, that the Nova Scotia Department of Trade and Industry, through its Inland Fisheries Survey, provided the necessary field collecting and measuring equipment.

We are indebted to Mr. James Lewin of the Inland Fisheries Survey for surveying the lake, and for much assistance with the field work. Miss Shirley Mason conducted a botanical examination of the lake, and Messrs. D. A. Livingstone and M. L. Cameron also aided with the field work. Messrs. I. H. S. Henderson and I. I. Tingley assisted with the preparation and analysis of samples. The advice of Dr. W. J. Chute and Dr. H. P. Bell was frequently sought, and very generously given.

Without the assistance of friends, particularly those mentioned, it would have been impossible to carry through the work.

DISTRIBUTION OF P^{32} IN CHICK EMBRYOS[1]

By J. B. O'Neil,[2] J. R. Jowsey,[3] C. C. Lee,[4] and J. W. T. Spinks[5]

Abstract

By feeding a hen repeated daily doses of sodium dihydrogen phosphate of equal P^{32} activity, eggs with high and nearly uniform P^{32} content were obtained for use in hatching experiments. Eleven eggs were incubated. The embryos and chicks that died during or after incubation were dissected and analyzed for total and specific activities in the various fractions. Two of the chicks, both females, are still living. They are being raised so that observations may be made on their ability to reproduce and the effect of the radioactivity, if any, on their offspring.

Introduction

A study by Plimmer and Scott (8) of the transformation of the phosphorus compounds in the egg to the phosphorus compounds in the chick showed that vitellin disappeared at the end of incubation. There was also a pronounced shift from a large amount of ether-soluble phosphorus and a trace of inorganic phosphorus in the egg to a relatively small amount of ether-soluble phosphorus and a large amount of inorganic phosphorus in the chick. Such results indicate that hydrolysis of organic phosphorus compounds to the inorganic form must take place during incubation.

The metabolism of lipoid phosphorus in the chick embryo has been studied by Kugler (5). The large store of phosphatides in the yolk, even shortly before the egg is hatched, as observed by Kugler (5), may lead one to suppose that the embryo might utilize these phosphatides directly in the building up of organs that contain such substances. The validity of this supposition has been tested by Hevesy *et al.* (4) by incubating eggs, each of which had received an injection into the white of P^{32}-labeled inorganic phosphate. It was found that at various stages of incubation, the phosphatide phosphorus extracted from the embryo always showed high specific activity while that extracted from the yolk was hardly active at all. The phosphatide molecules present in the embryo could not, therefore, have been taken from the yolk only, but must have been synthesized in the embryo.

The distribution of P^{32} in incubated eggs has also been studied by Dixon (2). This author administered the activity by injection of labeled potassium dihydrogen phosphate into the yolks of fertile eggs that had been incubated for five days. From the sixth day of incubation to the day of hatching, the P^{32} in the embryo was found to increase from 3% to 90% of the dose injected. The high activity observed in the allantoic and amniotic fluids showed that

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within 24 hr. following injection a shift of P^{32} from the yolk to the allantois and amnion takes place, and, as the embryo grows, the rate of uptake of phosphate from the allantois and amnion is much greater than from the yolk.

The injection of labeled phosphate into the egg, as employed by Dixon (2) and by Hevesy *et al.* (4), affords a convenient means for studying the role played by the phosphorus of inorganic phosphate in the metabolism of the chick embryo. It is well known, however, that P^{32} may be incorporated into the phosphorus compounds of the egg by administration of labeled phosphate to the hen (1, 7). The hatchability of such eggs has, as yet, not been studied. Recently, Spinks *et al.* (10) showed that after they fed a hen repeated daily doses containing an equal amount of P^{32}, for two weeks, the activity in the eggs became practically constant. This provides an excellent method for obtaining eggs of high and nearly constant activity for hatching experiments. This communication reports the results of such experiments with eggs from a hen that had received 40 daily doses of equal P^{32} activity.

Experimental

A Barred Plymouth Rock hen was isolated in a separate pen for the experiment. For a period of 40 days, the daily breeding mash was mixed with a solution of sodium dihydrogen phosphate containing 9450 registers per minute* (2.12 mc.) of P^{32} activity and fed to the hen in the morning. The decay of the P^{32} was taken into account by increasing the volume of the active solution so that the same amount of activity was fed every day. In the afternoons, when all the feed had been consumed, a Barred Plymouth Rock male was placed in the pen. Three males were used alternately throughout the 40 day period so as to ensure fertility of the eggs.

The eight eggs laid in the first two weeks following the first feeding were analyzed for total activity in the white + yolk. This involved the wet ashing of an aliquot of the white + yolk with nitric and perchloric acid, precipitation of the phosphorus as the phosphomolybdate, conversion to magnesium ammonium phosphate, and final ignition to magnesium pyrophosphate before the activity was counted. The detailed procedure has been reported previously (10). The total phosphorus was also determined, using a small aliquot of each ash solution, according to the colorimetric method of Shelton and Harper (9).

Eleven more eggs, all of which were fertile, were laid between the 15th and 46th day after the first feeding. These were incubated under standard conditions. The chicks that died during or after incubation were dissected into various fractions, as shown in the accompanying tables; these were wet ashed and the total activity and total phosphorus were determined for each fraction.

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* *One register per minute on the mechanical counter is equal to 128 radioactive disintegrations counted. The actual number of beta disintegrations is equal to the number of counts multiplied by a factor of 3.9 (counter efficiency was measured using an RaD–RaE standard from the National Bureau of Standards, Washington).*

When necessary, 5 to 10 mgm. of inactive ammonium phosphate were added to the aliquot being used for the determination of radioactivity to act as carrier before the phosphorus was precipitated. From the two chicks that are normal and still living, droppings were collected, twice a week, wet ashed, and the radioactivity and total phosphorus found. The analysis of the droppings was discontinued when the activity had decayed to a small value.

Results

The results of the analyses of the white + yolk of the first eight eggs as well as the total recovery of P^{32} from all fractions of the embryos or chicks are given in Table I. All activities tabulated in this and other tables in this

TABLE I

TOTAL RECOVERY OF P^{32} FROM UNINCUBATED AND INCUBATED EGGS

Column I is the time of oviposition in hours after the first feeding of P^{32}.
Column II is the total activity as of the day of oviposition in reg. per min.
Column III is the total recovery expressed as % of the daily P^{32} dosage.
Column IV is the total phosphorus in mgm.

Egg No.	I	II	III	IV	Remarks
1	9	2	0.02	73.4	White + yolk analyzed
2	45	31	0.33	83.0	"
3	70	149	1.58	79.6	"
4	172	756	8.00	76.4	"
5	195	819	8.66	77.8	"
6	222	837	8.85	80.1	"
7	269	980	10.35	81.0	"
8	313	966	10.22	87.4	"
9	413	971.6	10.27	71.28	Embryo died on about the 18th day of incubation
10	528	1050.3	11.10	68.89	Embryo died on about the 18th day of incubation
11	556	964.6	10.20	73.89	Chick killed on the day of hatching
12	606	1048.4	11.08	73.58	Chick died on the day of hatching
13	646	1048.7	11.08	67.35	Chick died two days after hatching
14	701	Lost on wet ashing			Chick died on the day of hatching
15	886	1017.8	10.77	83.60	Embryo died on about the 7th day of incubation
16	912				Chick living
17	982				Embryo died on about the 18th day of incubation—used for preliminary trials in radioautography
18	1009	1004.2	10.63	83.32	Chick died on the day of hatching
19	1108				Chick living

paper are based on the day of oviposition, correction factors having been applied to allow for the decay of the activity from the day of oviposition to the day of analysis. The total recoveries shown in Table I are also expressed as % of the daily dosage of P^{32} fed and are plotted in Fig. 1. From Fig. 1, it may be seen that the total activity in each of the incubated eggs on the day of oviposition was in the order of 11% of the daily P^{32} dosage.

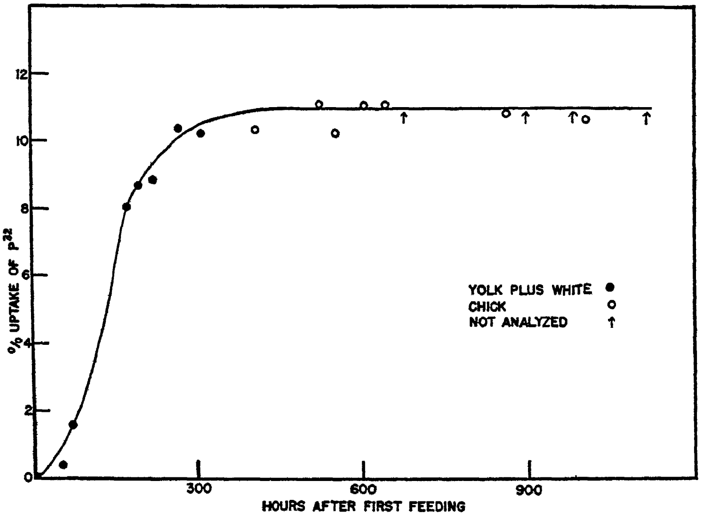

FIG. 1. *Recovery of P^{32} in successive eggs (or chicks) expressed as a percentage of the P^{32} fed on any one day. Same activity of P^{32} fed as sodium dihydrogen phosphate for 40 successive days. (Chicks not analyzed indicated by an arrow.)*

All of the 11 eggs (No. 9 to 19) incubated were fertile. The first chick that hatched was from egg No. 11. It showed no apparent abnormalities. It was killed and dissected on the day of hatching. Two other chicks, hatched from eggs Nos. 16 and 19, were normal and healthy; these are still living. Results of the analyses of their droppings are tabulated in Table II and plotted in Fig. 2.

The embryo from egg No. 15 died on about the seventh day of incubation. Results obtained from this egg are given in Table III. After incubation for about 18 days, the embryos from eggs Nos. 9, 10, and 17 died. Of these, the embryo from egg No. 17 was used for preliminary trials in radioautography. Those from eggs Nos. 9 and 10 were analyzed and the results are tabulated in Table IV. The ages of these four embryos were approximated by comparing the state of their development with diagrams given in Ewing's *Handbook of Poultry Nutrition* (3).

The chicks from eggs Nos. 12, 14, and 18 died on the day of hatching. Some of the fractions of the chick from egg No. 14 were lost during wet ashing.

TABLE II

ACTIVITY OF DROPPINGS OF CHICKS HATCHED FROM EGGS NOS. 16 AND 19—CHICK FROM EGG NO. 16 WAS HATCHED ON SEPT. 4, 1948; CHICK FROM EGG NO. 19 WAS HATCHED ON SEPT. 14, 1948

Column I is the period during which the sample was collected, expressed in days after hatching.
Column II is the P^{32} activity as of the day of oviposition in reg. per min.
Column III is the total phosphorus in mgm.
Column IV is the specific activity as of the day of oviposition in reg. per min. per mgm. P.

Chick from egg No. 16				Chick from egg No. 19			
I	II	III	IV	I	II	III	IV
0- 7	71.2	50.8	1.40	0- 8	74.5	41.6	1.79
8–11	96.4	47.2	2.04	9–12	50.8	71.5	0.71
12–15	88.0	62.8	1.40	13–15	22.4	66.2	0.34
16–18	49.0	57.2	0.86	16–19	36.0	63.2	0.57
19–22	66.5	70.3	0.95	20–22	22.6	69.4	0.33
23–25	42.2	68.8	0.64	23–26	32.6	70.3	0.46
26–29	39.6	73.8	0.54	27–29	32.8	66.1	0.50
30–32	26.2	67.5	0.39	30–33	19.3	71.5	0.27
33–36	34.7	71.5	0.49	34–36	12.3	64.2	0.19
37–39	28.2	68.2	0.41				
40–43	29.4	70.3	0.42				
44–46	21.7	70.5	0.31				

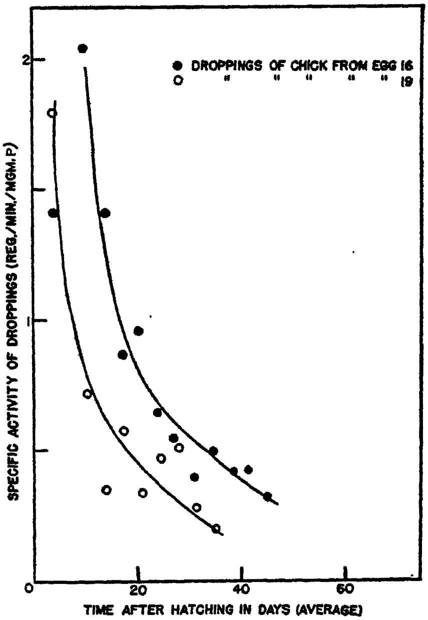

FIG. 2. *Specific activity of droppings from radioactive chicks plotted against time after hatching.*

TABLE III

ACTIVITY OF EMBRYO FROM EGG NO. 15—EMBRYO DIED ON ABOUT THE SEVENTH DAY OF INCUBATION

Column I is the activity of the corresponding fraction as of the day of oviposition in reg. per min.
Column II is the activity expressed as % of the total recovered activity.
Column III is the total phosphorus in mgm.
Column IV is the specific activity of the corresponding fraction as of the day of oviposition in reg. per min. per mgm. P.
Column V is the ratio of the specific activity of the fraction to the overall specific activity.

Fraction	I	II	III	IV	V
Viscera	3.5	0.34	0.11	31.80	2.62
Carcass	8.2	0.80	0.55	14.90	1.22
Embryonic fluids and membranes	14.1	1.38	1.04	13.55	1.11
Yolk and white	992	97.48	81.90	12.11	0.996
Total	1017.8	100.00	83.60		

Overall specific activity 1017.8/83.60 = 12.16 reg. per min. per mgm. P

TABLE IV*

ACTIVITY OF EMBRYOS FROM EGGS NOS. 9 AND 10—EMBRYOS DIED ON ABOUT THE 18TH DAY OF INCUBATION

Fraction	Embryo from egg No. 9					Embryo from egg No. 10				
	I	II	III	IV	V	I	II	III	IV	V
Liver	11.6	1.19	1.17	9.92	0.73	8.2	0.78	0.86	9.54	0.63
Brain	13.4	1.38	1.28	10.40	0.76	13.3	1.27	1.71	7.78	0.51
Heart	3.1	0.32	0.34	9.12	0.67	1.5	0.14	0.21	7.15	0.47
Gizzard muscles	7.3	0.75	0.92	7.94	0.58	6.6	0.63	0.91	7.25	0.48
Skin and feathers	31.7	3.26	2.56	12.38	0.91	46.8	4.46	3.06	15.30	1.00
Leg muscles	19.3	1.99	2.05	9.42	0.69	23.9	2.27	2.11	11.33	0.74
Tibiae	35.3	3.64	3.81	9.27	0.68	49.1	4.68	5.10	9.63	0.63
Remainder of viscera	15.9	1.64	1.65	9.65	0.71	24.9	2.37	2.23	11.16	0.73
Remainder of carcass	356	36.63	26.38	13.50	0.99	437	41.60	26.00	16.80	1.10
Yolk sac	358	36.84	27.70	12.92	0.95	439	41.80	26.70	16.43	1.08
Embryonic fluids and membranes	120	12.35	3.42	35.10	2.57					
Total	971.6	100.00	71.28			1050.3	100.00	68.89		
Overall specific activity	971.6/71.28 = 13.63 reg. per min. per mgm. P					1050.3/68.89 = 15.27 reg. per min. per mgm. P				

* *For explanation of column numbers, see Table III.*

Consequently, the analysis of this chick was not completed. The results of the analyses of the chicks from eggs Nos. 12 and 18, together with those of the chick from egg No. 11, are tabulated in Table V.

TABLE V*

ACTIVITY OF CHICKS FROM EGGS NOS. 11, 12, AND 18—CHICK FROM EGG NO. 11 WAS KILLED ON THE DAY OF HATCHING, CHICKS FROM EGGS NOS. 12 AND 18 DIED ON THE DAY OF HATCHING

Fraction	Chick from egg No. 11					Chick from egg No. 12					Chick from egg No. 18				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Blood	11.7	1.21	1.31	8.93	0.69	20.0	1.91	1.58	12.66	0.89	—	—	—	—	—
Liver	16.8	1.74	1.91	8.80	0.68	27.0	2.57	1.61	16.77	1.18	12.4	1.24	1.47	8.44	0.70
Brain	18.7	1.94	1.94	9.65	0.74	22.4	2.14	1.75	12.80	0.90	17.7	1.76	2.04	8.68	0.72
Heart	3.5	0.36	0.56	6.25	0.48	4.9	0.47	0.36	13.60	0.96	4.3	0.43	0.46	9.35	0.78
Gizzard muscles	17.0	1.76	1.75	9.73	0.75	21.7	2.07	1.91	11.36	0.80	15.4	1.53	1.76	8.76	0.73
Skin and feathers	23.4	2.42	1.91	12.25	0.94	31.6	3.01	2.68	11.80	0.83	31.3	3.12	2.97	10.53	0.87
Leg muscles	39.0	4.04	4.53	8.61	0.66	44.2	4.22	2.68	16.50	1.16	40.2	4.00	3.13	12.84	1.06
Tibiae	54.3	5.63	6.10	8.90	0.68	74.0	7.05	5.63	10.50	0.74	64.1	6.39	7.25	8.58	0.73
Remainder of viscera	30.2	3.13	3.00	10.06	0.77	31.1	2.96	2.58	12.05	0.85	16.8	1.67	1.88	8.95	0.74
Remainder of carcass	472.5	49.0	32.0	14.75	1.13	521	49.7	34.25	15.22	1.08	518	51.56	34.3	15.10	1.25
Yolk sac	277.5	28.77	18.88	14.70	1.13	250	23.9	18.55	13.50	0.95	284	28.30	28.06	10.12	0.84
Total	964.6	100.00	73.89			1048.4	100.00	73.58			1004.2	100.00	83.32		
Overall specific activity	964.6/73.89 = 13.04 reg. per min. per mgm. P					1048.4/73.58 = 14.24 reg. per min. per mgm. P					1004.2/83.32 = 12.06 reg. per min. per mgm. P				

* *For explanation of column numbers, see Table III.*

The chick from egg No. 13 died two days after hatching. Results of its analysis are given in Table VI.

TABLE VI*

ACTIVITY OF CHICK FROM EGG NO. 13—CHICK DIED TWO DAYS AFTER HATCHING

Fraction	I	II	III	IV	V
Liver	22.3	2.13	2.03	10.98	0.71
Brain	20.4	1.94	1.80	11.32	0.73
Heart	7.7	0.73	0.44	17.49	1.12
Gizzard muscles	20.2	1.93	2.45	8.25	0.53
Skin and feathers	42.3	4.03	3.36	12.60	0.81
Leg muscles	43.7	4.17	3.31	13.20	0.85
Tibiae	59.8	5.70	3.78	15.80	1.01
Remainder of viscera	36.4	3.47	2.43	14.97	0.96
Remainder of carcass	583	55.60	29.50	19.75	1.27
Yolk sac	196	18.69	16.80	11.67	0.75
Droppings	16.9	1.61	1.45	11.65	0.75
Total	1048.7	100.00	67.35		
Overall specific activity	1048.7/67.35 = 15.57 reg. per min. per mgm. P				

* *For explanation of column numbers, see Table III.*

Discussion

Of the 11 fertile eggs incubated, one embryo died on the seventh day of incubation, three embryos died on the 18th day of incubation, three chicks died on the day of hatching, one chick died two days after hatching, one apparently normal chick was sacrificed on the day of hatching, and two apparently normal chicks are still living. The mortality, although very high, could still be considered within the limit observed for ordinary eggs since in the average mortality curve a maximum is reached on about the 18th day of incubation. The fact that two of the chicks could be kept alive indicates that, under the conditions of the experiments reported in this paper, the radioactivity has no apparent effect on the hatching of the egg and the normal growth of the chick. These two chicks are females. They are being raised so that observations may be made on their ability to reproduce and the effect of the radioactivity, if any, on their offspring. Inspection of the amount of P^{32} excreted by these two chicks (Table II) indicates that the chick from egg No. 19 probably contained much less radioactivity. This is not surprising since egg No. 19 was laid six days after the termination of the daily feeding of P^{32}.

From the embryos and chicks that were analyzed, radioactivity was found in all fractions examined. As is to be expected, the greatest amount of activity was found in the tissues that contain the largest quantity of phosphorus. Thus the fraction denoted "remainder of carcass", which consisted of all the bones except the tibiae and all the flesh except the leg muscles, contained about 40% of the total activity recovered from the embryos that died on the 18th day of incubation, about 50% of the total activity recovered from the chicks that died on the day of hatching, and about 55% of the total activity recovered from the chick that died two days after hatching. The P^{32} in the yolk sac decreased from approximately 40% of the total activity in the embryos that died on the 18th day of incubation to less than 20% in the two-day-old chick. This is compatible with the well known fact that the yolk sac is rapidly absorbed during the first few days of life of the chick.

The specific activity (registers per min. per mgm. P) and the ratio of the specific activity of the fraction to the overall specific activity (the overall specific activity is the sum of activities divided by the sum of total phosphorus for all fractions of a given chick) as well as the distribution of the activity as a % of the total activity recovered are tabulated in Tables III, IV, V, and VI. A low value for the ratio of the specific activity of a given fraction to the overall specific activity is indicative of a dilution of the radiophosphorus. It is interesting to note that of all the fractions of the embryo from egg No. 9, which died on the 18th day of incubation, the embryonic fluids and membranes fraction has the highest specific activity. In the dissection of the embryo from egg No. 10, which also died on the 18th day of incubation, the yolk sac was accidentally broken and its content mixed with the embryonic fluids. However, the combined yolk sac plus embryonic fluids and membranes fraction also showed a relatively high specific activity. In a study of the relative abundance of different acid soluble phosphorus compounds in the embryonic fluid, Kugler (6) concluded that there is a high inorganic phosphorus content in the allantoic fluid. This finding, coupled with the observed high specific activity of the embryonic fluids and membranes fraction and the high rate of uptake of phosphate from the allantois and amnion found by Dixon (2), suggest that phosphorus compounds of high specific activity present in the egg, such as vitellin (1), may be hydrolyzed to inorganic phosphate, transported to the allantois and thence absorbed by the embryo. Such a mechanism would be in accord with the conclusion of Hevesy *et al.* (4) that practically all phosphorus atoms present in the various compounds of the embryo must pass through the stage of inorganic phosphorus.

With the limited number of chicks analyzed, no final interpretation can be made of the P^{32} distribution and the specific activities in the liver, brain, heart, etc. More experiments will be carried out on the distribution of P^{32} in chicks of different ages and in embryos at various stages of development.

Acknowledgment

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A KEY TO THE EMBRYOLOGICAL DEVELOPMENT OF *MELANOPLUS BIVITTATUS* (SAY), *M. MEXICANUS MEXICANUS* (SAUSS.), AND *M. PACKARDII* SCUDDER[1]

By R. W. Salt[2]

Abstract

The embryological development of three species of grasshoppers is described in gross aspect by means of a key that relates morphological development to the duration of incubation at 25° C. The three species are so similar that they are treated as one. The rate of development and stage of entering diapause are compared with those of *M. differentialis*.

A comprehensive illustrated account of the embryological development of *Melanoplus differentialis* (Thos.) at 25° C. was published by Slifer in 1932 (2). In 1936 Steele (4) published a briefer account of development in embryos of *Austroicetes cruciata* Sauss. Possibly as a result of some similarity between these two species and the popularity of *M. differentialis* eggs as laboratory test animals, it is often assumed that their eggs are representative of acridid eggs generally. Moore (1), however, points out that both *M. bivittatus* and *M. mexicanus mexicanus* differ from *M. differentialis* in respect to embryonic development and diapause. In the present study three species of *Melanoplus* were found to be so similar to each other in embryological development as to be virtually the same, yet greatly different from that reported for *M. differentialis* and *A. cruciata.* The purpose of this paper, however, is not so much to point out differences among species as to establish a convenient means of reference to embryonic development similar to that used by Slifer (2), for use in further studies.

Materials and Methods

Newly laid eggs of *Melanoplus bivittatus*, *M. m. mexicanus*, and *M. packardii* were obtained from caged adults and incubated on moist plaster of Paris at 25° C. The younger embryos were dissected from the eggs for observation but the older ones were observed *in situ* and *in vivo*, using the excellent method of chorion removal described by Slifer (3).

Results

The accompanying key makes use of morphological characters by which the course of development can be transposed into time of incubation at 25° C. From the fourth day of incubation onwards these characters are readily observable under a binocular dissecting microscope. During the first three days the embryos are too small for this method, and therefore the first three days are omitted.

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Although the three species exhibited minor differences of size and appearance there was no observable difference in development or its rate. For the purpose at hand, therefore, they may be treated as one.

Key to the Development of Embryos of *Melanoplus bivittatus*, *M. m. mexicanus*, and *M. packardii* at 25° C.

"Tail" about same length as diameter of head	4 days
"Tail" about twice as long as head; no other differentiation into body regions	5 days
"Tail" about three times as long as head; body vaguely differentiated into four regions	6 days
Differentiation into protocephalic, protognathal, thoracic, and abdominal regions distinct; no detail in head	7 days
Antennae, labrum, mouth parts, and legs appear as distinct knobs; no abdominal segmentation	8 days
Segmentation appearing in mouth parts and legs; abdomen distinctly segmented, with appendages appearing as vague knobs; labrum not notched	9 days
Labrum notched; abdominal appendages appear as distinct knobs, with first pair (pleuropodia) larger but not greatly dissimilar to the others	10 days
Pleuropodia trilobed; other abdominal appendages still knoblike; no antennal segmentation	11 days
Hind legs beginning to fold into N-shape	12 days
Hind legs folded into N-shape; distal half of antennae shallowly segmented	13 days
Posterior rim of eyes faintly pink; embryo half the length of egg but narrow; thorax one-third width of egg. Anatrepsis now completed	14 days
Revolution of the embryo in progress; red in eyes forms thin crescents	15 days
Revolution completed; head pointing anteriorly; embryo half the length of egg, thorax three-quarters of egg width (16- to 20-day stages identifiable through the wet chorion by location, size, and coloration of the eyespots)	16 days
Embryo two-thirds of egg length; eyespots more distinct	17 days
Embryo three-quarters of egg length; yolk engulfment advanced	18 days
Yolk engulfment and dorsal closure complete; embryo full length; eyes red on upper margins only; hind femora reaching Abd. IV or V	19 days
Eyes red on half of surface; hind femora reach Abd. VI or VII	20 days
Eyes dark red on all but lower rim; mandibular teeth, lacinial teeth, and tarsal claws unpigmented and difficult to see	21 days

(DIAPAUSE OCCURS HERE IF AT ALL)

Mandibular teeth pigmented enough to be distinctly seen; eye pigmentation complete	22 days
Mandibular teeth dark piceous; lacinial teeth, tarsal claws, and rows of spines on hind tibiae lightly pigmented	23 days
General pigmentation darker; all spurs, spines, and teeth dark piceous to black; morphological detail complete; apparently ready to hatch	24 days

The relationship between development and time as given in the key should not be interpreted as rigid or precise, but rather as an optimum. Though most embryos developed on schedule up to the onset of diapause, many fell behind for unknown reasons. At least some of this retardation is thought to be caused by handling and other artificialities of laboratory rearing. The postdiapause period is described as three days. Actually the measurement of this period is made uncertain by the difficulty of determining exactly when diapause is ended, or more specifically, when postdiapause development begins. From observation of developing embryos in which diapause was of very short duration, a postdiapause period of about three days, during which time pigmentation steadily increased, seemed to be about optimal. However, hatching does not necessarily take place as soon as the embryo reaches the 24-day stage. It appears in most cases that a stimulus to hatch may be necessary; if so, its nature is not known.

Discussion

In comparison with *Melanoplus differentialis* (2), two differences are outstanding. The rate of development of *M. differentialis* at 25° C. is much slower and diapause occurs at a much earlier stage. In 21 days *M. differentialis* has completed anatrepsis and enters diapause. Embryos of *M. bivittatus*, *M. m. mexicanus*, and *M. packardii* complete anatrepsis in 14 days, or 1.5 times as fast as *differentialis*. They continue development and enter diapause on the 21st day at a developmental stage far beyond that of *differentialis*.

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WATER UPTAKE IN EGGS OF *MELANOPLUS BIVITTATUS* (SAY)[1]

BY R. W. SALT[2]

Abstract

Eggs of *Melanoplus bivittatus* (Say) possess a special water-absorbing area, the hydropyle, similar in function to that of *M. differentialis* (Thos.). The extent of hydration was measured as eggs of *M. bivittatus* developed at 25° C. on contact moisture. The total amount of water absorbed amounts to about 60% of the original weight of the egg. Absorption is negligible for the first four days, is maximal on the eighth and ninth days, and later may exhibit a second period of minor increase and decline, which varies in time of occurrence. About 88% of the total absorption occurs during anatrepsis. Eggs denied contact moisture from the time of oviposition develop to the end of anatrepsis and remain viable for long periods, provided desiccation is not severe. When contact moisture is provided, such eggs hydrate very rapidly and continue development with a retardation of two or three days. Extra moisture from the environment is essential for the revolution and katatrepsis of the embryo.

Introduction

Eggs of *M. bivittatus* absorb water from the environment. The site and extent of absorption, and its influence on embryological development, constitute the studies reported here.

The Site of Absorption

The presence of a hydropyle such as described by Slifer (6) was verified for *M. bivittatus*. Following the method found most satisfactory by her, the posteriors of 20 eggs were coated with "liquid solder". Twenty eggs with the anterior ends coated and 10 untreated eggs served as controls. All of the eggs were from the same pod, which had been kept at 25° C. and 90% R.H. for 20 days following oviposition. They were thus denied contact moisture and had ceased development at the end of anatrepsis.

After two days on moist filter paper the untreated eggs and those with the anterior ends sealed were noticeably swelled; by the fourth day all of them appeared fully hydrated and on the fifth day the eye spots showed each egg to contain a 16- or 17-day embryo. As the solder held its shape, the eggs treated at the anterior end swelled posteriorly until it seemed they would burst. They continued healthy for some time and either hatched or entered diapause. Meanwhile the eggs sealed at the posterior or micropyle end showed no change in size or shape for six days. The solder was then softened with ethyl acetate and scraped off, after which the chorions were removed in sodium hypochlorite (7). When these 20 eggs were exposed to contact moisture (vertical, hydropyle-down, to ensure contact in the absence of the moisture-conducting chorion) nine promptly hydrated and developed. Failure of the remainder to develop was attributed to injury in removing the solder.

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Although transpiration through the general cuticular surface is not disproved, it would appear to be very limited if not absent. The main site of absorption is the micropylar area, as already shown in *M. differentialis* (6) and *Rhodnius prolixus* Stal. (1).

The Course of Absorption During Embryological Development

Isolated newly laid eggs were incubated on moist filter paper at 25° C. Each egg was weighed within 24 hr. of oviposition and every second day thereafter for 20 days on a torsion spring balance (150/0.1 mgm). On the 16th, 18th, and 20th days the age of each egg was determined by observing the position of the eye spots. Because weight increase was to be related to development, only those eggs that developed on schedule (4) are included in the analysis. On the basis of Bodine's observations on *M. differentialis* (3) it is assumed that weight increase equals water uptake.

The average weights of each group of eggs are listed in Table I and the accumulation percentage increases in Table II. As the variance among eggs of the same pod was not significant, only the means are listed in Table I.

TABLE I

Mean weights in mgm. of eggs developing on schedule at 25° C. on contact moisture

Series	No. of eggs	Days										
		0	2	4	6	8	10	12	14	16	18	20
A	12	4.12	4.14	4.18	4.38	5.30	6.18	6.37	6.52	6.55	6.67	6.78
B	13	4.11	4.14	4.18	4.42	5.15	5.93	6.30	6.35	6.49	6.55	6.59
C	9	3.77	3.77	3.77	3.97	4.80	5.74	5.88	5.94	6.06	6.17	6.17
D	10	4.12	4.12	4.14	4.16	4.94	5.86	6.21	6.32	6.41	6.47	6.47
E	7	4.34	4.34	4.34	4.57	5.39	6.29	6.57	6.71	6.81	6.93	6.94
F	8	4.61	4.62	4.69	4.85	5.81	6.50	6.59	6.64	6.85	6.96	6.98
G	22	4.86	4.86	4.86	5.30	6.20	7.10	7.37	7.47	7.70	7.76	7.81
H	47	3.84	3.84	3.85	3.94	4.68	5.43	5.77	6.00	6.10	6.17	6.23
Mean		4.16	4.17	4.19	4.38	5.18	5.99	6.30	6.44	6.57	6.65	6.69

The same data are presented graphically in Fig. 1, in which it should be noted that the time axis is simultaneously embryonic development and days of exposure to contact moisture, because all eggs developed on schedule. Water uptake is negligible for about four days, after which it increases rapidly to a maximum on the eighth and ninth days, followed by a rapid decline. In seven of the eight groups a second, minor increase in absorption rate also appears. This is not uniform in point of time, however, with the result that combining the data of all groups (Fig. 1, I) obscures the second mode. The second rate increase does not appear to be related directly to embryonic age although it may be related indirectly as a characteristic of the micropylar absorption area.

TABLE II

MEAN ACCUMULATIVE PERCENTAGE WEIGHT INCREASES OF EGGS DEVELOPING ON SCHEDULE AT 25° C. ON CONTACT MOISTURE

Series	No. of eggs	Days									
		2	4	6	8	10	12	14	16	18	20
A	12	0.5	1.5	6.3	28.7	50.0	54.7	58.3	59.0	61.9	64.6
B	13	0.7	1.7	7.5	25.3	44.3	53.3	54.5	57.9	59.4	60.3
C	9	0.0	0.0	5.3	27.3	52.2	56.0	57.6	60.8	63.6	63.6
D	10	0.0	0.5	1.0	19.9	42.2	50.8	53.6	55.6	57.1	57.1
E	7	0.0	0.0	5.3	24.2	44.9	51.4	54.6	56.9	59.7	59.9
F	8	0.2	1.7	5.2	26.0	41.0	43.0	44.0	48.6	51.0	51.4
G	22	0.0	0.0	9.1	27.6	46.1	51.7	53.7	58.5	59.7	60.7
H	47	0.0	0.3	2.6	21.9	41.4	50.2	56.2	58.9	60.7	62.2
Mean		0.2	0.7	5.3	24.5	45.2	51.5	54.8	58.0	59.9	60.8

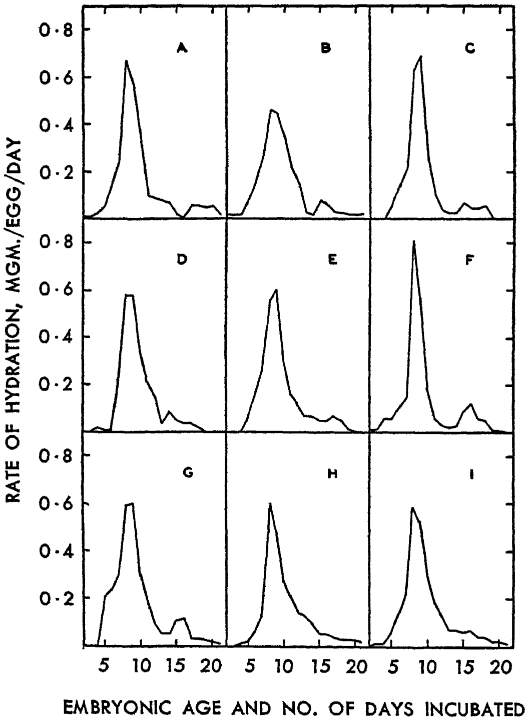

FIG. 1. *Rates of hydration in milligrams per egg per day of eggs developing on schedule at 25° C. on contact moisture. A to H—means of samples from single pods; I—mean of all eggs.*

The proportional increase in weight of the eggs bulked in one group may be followed in Fig. 2. The period of greatest absorption lies between the 6th and 12th days, during the latter part of anatrepsis, but without any apparent relationship to embryonic growth. The dotted line represents the theoretical levelling-off of weight had the second absorption period been absent; the actual increase is thereby accentuated.

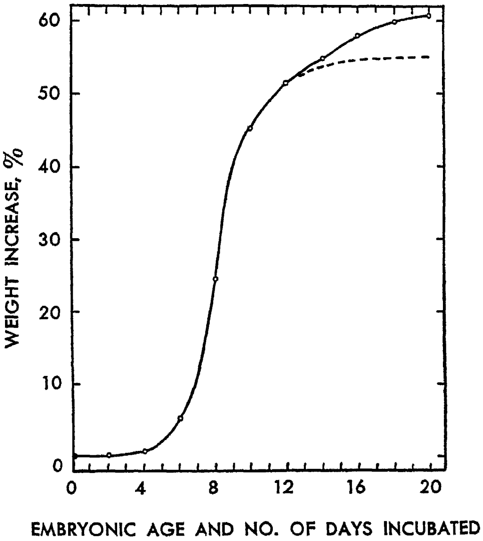

FIG. 2. *Mean percentage weight increase of 128 eggs developing on schedule at 25° C. on contact moisture. Solid line—observed data; dotted line—theoretical position if second absorption period were not present.*

The experimental conditions described above allowed continuous, maximum absorption. In order to determine how necessary this is to the egg and to the embryo, experiments limiting the water supply were carried on. It was first established that eggs exposed to 25° C. and 50% R.H. from the time of oviposition survived for at least 20 days, during which time they completed anatrepsis but never developed further. In order to avoid both excessive dehydration and contact moisture, large numbers of eggs were placed at 25° C. and 90% R.H. Over 90% of these eggs completed anatrepsis and remained viable for at least 60 days, some living as long as 200 days. Here again, as at 50% R.H., development ceased at the end of anatrepsis. When contact moisture was supplied to such eggs they absorbed it very quickly. Table III shows that 92 such eggs absorbed nearly 50% of their weight in two days and in six days had gained as much as normally-incubated eggs. The hydration is so fast that the bimodal characteristic noted in Fig. 1 is not discernible.

Age records were kept for the eggs represented in Table III, which were handled as individuals, as well as for several hundred others that were not weighed. Most of the embryos reached the 16-day stage on the fourth day of hydration and practically all of the remainder by the fifth day. Thus the average retardation is somewhat over two days.

TABLE III

WEIGHT INCREASE AND DEVELOPMENT OF 92 EGGS ON CONTACT MOISTURE AT 25° C. AFTER 30 DAYS AT 25° C. AND 90% R.H. FROM DATE OF OVIPOSITION

	Days hydrated					
	0	1	2	3	4	6
Average weight, mgm.	4.50	5.80	6.70	6.95	7.03	7.20
Accumulative increase, %	—	28.9	48.9	54.5	56.2	60.0
Predominant embryonic age	14	—	—	—	16	18

It might be supposed that the weight of the egg would give a rough indication of its embryonic development. This is far from being the case. Variation in weight was found to be very great for any age group, even for eggs taken from the same pod. Including eggs up to five days of age, by which time hydration is negligible, 1856 eggs from 85 pods ranged from 3.0 mgm. to 5.5 mgm., each extreme being represented in newly laid eggs. Comparable variations are found throughout the range of embryonic growth. Thus, although hydration results in large weight increases, these may be obscured by normal size variation.

Discussion

In 1929 Bodine (3) published smoothed curves showing that eggs of *M. differentialis* absorb water during their development. No details were given regarding amounts, but it was indicated that there was a fairly steady increase in weight and water content during the active developmental period. The increase was interrupted by diapause, during which time a steady level of water content was maintained. In 1938 Slifer (6) published curves of prediapause and postdiapause weight increases for the same species. These represent averages of large numbers of eggs and reveal that absorption is very slight during the first week. This is in agreement with findings for *M. bivittatus* when a correction is made for the differences in rate of development. (*M. differentialis* reaches the end of anatrepsis in 21 days at 25° C. (5); *M. bivittatus* in 14 days. Hence the latter's rate of development is about 1.5 times that of *M. differentialis.*) By the time anatrepsis is complete, *M. differentialis* has absorbed about 0.7 mgm. per egg; *M. bivittatus* 2.28 mgm. (Table I). During katatrepsis, *M. differentialis* absorbs about 3.2 mgm.; *M. bivittatus* about 0.3 mgm. The pattern of absorption is thus entirely different in the two species; *differentialis* absorbs water chiefly during katatrepsis and *bivitattus* during anatrepsis.

It is of interest to compare the relationship between hydration and diapause in the two species. Slifer (8) has shown that in *M. differentialis* diapause can be broken by a 30-min. immersion in a fat solvent such as xylol, which acts by dissolving a lipid layer at the hydropyle and allowing the entrance of water. *M. bivittatus* is similar in that it also requires water from the environment before katatrepsis can proceed, but is different in that the absorption normally takes place during anatrepsis and has no connection whatsoever with diapause. We have seen that if young eggs of *M. bivittatus* are denied contact moisture they cease development at the end of anatrepsis, which is the stage at which *M. differentialis* enters diapause. They cannot develop further until they have absorbed water, and if this is not forthcoming they survive for rather long periods but are ready at any time to absorb water and resume development. Thus they are not in a state of true diapause although the result is similar. The condition is comparable to that of a chilled insect that is able to resume normal activity and development as soon as it is warmed.

Although Slifer warns against the assumption that hydration is the only factor involved in the resumption of growth in *M. differentialis*, it is surely a very important one. In contrast, it is difficult to see how hydration could possibly play a part in diapause in *M. bivittatus* eggs. In this species diapause starts on the 21st day at 25° C. (corresponding to about 32 days for the slower *M. differentialis*). By this time hydration is complete or practically so, and the embryo is almost completely developed. Nor does the bimodal character of the absorption curve bear any apparent relation to diapause. Though variable in its time of occurrence, the second mode occurs at least several days before the onset of diapause. Slifer's postdiapause absorption curve (6) does not give any indication of being bimodal; if actually so, it is obscured by time variation as in Fig. 1, I and Fig. 2.

Birch and Andrewartha (2) give brief records of the weight of eggs of *Austroicetes cruciata* Sauss. at certain stages of embryonic development. From these data it appears that hydration extended over the entire period and was about 40% completed by the end of anatrepsis. In the latter regard it differs from both *M. bivittatus* and *M. differentialis.* The same authors show that the eggs of *A. cruciata* absorb moisture during diapause. However, diapause in this species is much different from that in *Melanoplus*; it lasts during the entire course of anatrepsis except the first few days and may extend into katatrepsis also. Thus embryonic development progresses, though slowly, during diapause.

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PARASITES OF COD AND OTHER MARINE FISH FROM THE BAIE DE CHALEUR REGION[1]

BY ANITA FOCHS HELLER[2]

Introduction

Stafford (33) was the first to undertake the study of parasites of marine fishes along the Canadian Atlantic coast. However, his paper on the "Trematodes from Canadian fishes" created a confusion in the taxonomic literature owing to his unsatisfactory descriptions. This situation was remedied by Miller (23) who re-examined and re-identified the specimens in Stafford's slide collection. Further work on Canadian fish trematodes was done by Cooper (3). In 1930, Bere (2) published a paper on "The parasitic copepods of the fish of the Passamaquoddy region". On the Pacific coast, trematodes were dealt with by McFarlane (20). Wardle (35, 36, 37) dealt with cestodes and has also considered the Hudson's Bay area. Kuitunen-Ekbaum (12) and Smedley (31) have described nematodes.

However, there is still a lack of knowledge concerning the distribution of known species and it seemed that a further contribution toward this end would be of value. The still insufficient amount of systematic and faunistic information available on the marine parasites of Canada's east coast lends additional interest to their study.

The host species examined in the present survey occur also along the Atlantic coast of the United States and, therefore, the numerous publications of American authors have a direct bearing on the subject.

Because of the circumpolar distribution of the host fishes and their accompanying parasites, it has also been necessary to consult the results of European investigations.

Methods and Procedure

The codfish, skates, and flounders were taken on lines, partly three to four miles out at sea from Grande-Rivière, County Gaspé South, Quebec, Canada, and partly on the Miscou Bank about 30 mi. northeast of Grande-Rivière. The salmon were captured in salmon drift nets four miles out at sea. The

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herring were fished in drift nets three to four miles from shore and the mackerel were taken, accidentally, both in the salmon and herring nets. The dogfish came from the Miscou Bank, while the smelt were caught in dip nets a short distance up the Grande-Rivière, where they were swimming up river to spawn. The rest of the fish were caught, accidentally, in lobster traps near shore.

The host specimens were examined externally as soon as possible after their capture. The copepods were taken off the gills and skin and from inside the mouth cavity. The trematodes were collected from the gills and the oral cavity and the leeches were found inside the mouth. The body was then split open ventrally and the body cavity, muscles, and mesenteries were examined for nematodes and cestode larvae; the alimentary tract was lifted out from the oesophagus to the rectum, placed in a dish, and slit open. The contents were diluted with isotonic seawater and examined while still alive. The copepods were killed, fixed, and stored in 5% formalin. The leeches, nematodes, and acanthocephalans were killed, and fixed in hot 70% alcohol, and stored in 70% glycerine alcohol. The trematodes and cestodes were killed in hot fresh water and stored in 10% formalin.

In the fall, at the Institute of Parasitology, the trematodes and cestodes were stained, some with Ehrlich's acid haematoxylin, others with Gower's alum carmine. Both methods gave satisfactory results. They were then cleared in beechwood creosote and mounted in Canada balsam.

The Acanthocephala and Nematoda were cleared in lactophenol and examined, unmounted, while in that liquid. All the details, necessary for identification, were clearly visible by that method.

TABLE I

Hosts	Total number examined	Number of host specimens parasitized by:					
		Acanthocephala	Trematoda	Cestoda	Nematoda	Copepoda	Hirudinea
Acanthocottus scorpius (Shorthorn sculpin)	1	—	1	—	1	—	—
Clupea harengus (Herring)	27	—	11	—	2	—	—
Gadus callarias (Cod)	440	53	40	18	440	277	—
Microgadus tomcod (Tomcod)	1	1	1	—	1	—	—
Osmerus mordax (Smelt)	4	—	3	—	2	—	—
Pseudopleuronectes americanus (Winter flounder)	3	—	2	1	1	—	—
Salmo salar (Salmon)	5	—	3	4	2	4	—
Scomber scombrus (Mackerel)	3	—	—	—	3	—	—
Tautogolabrus adspersus (Cunner)	2	—	—	—	1	—	—
Urophycis chuss (Squirrel hake)	2	—	—	—	2	—	—
Zoarces anguillaris (Eelpout)	2	—	—	—	—	—	1
Raja erinacea (Summer skate)	2	—	—	2	2	—	—
Raja scabrata (Prickly skate)	20	—	1	13	1	2	—
Raja stabuliforis (Barndoor skate)	1	—	—	1	—	—	—
Squalus acanthias (Spiny dogfish)	3	—	—	—	—	—	—

Some of the Copepoda were removed from the formalin, washed, dehydrated, and cleared in lactophenol. Some specimens were washed and then heated in 10% sodium hydroxide in a water-bath so that the soft tissue was removed and only the chitinous structures remained. This latter procedure was used when a dissection of the mouth parts had to be carried out.

Results

(1) GENERAL

The accompanying table (Table I) shows how many specimens of each of the 15 host species were examined, as well as the number of these that were parasitized by the different groups of helminths or copepods.

(2) HELMINTHS

A. TREMATODA

Podocotyle atomon (Rudolphi, 1802) Odhner, 1905

Hosts: *Acanthocottus scorpius*, *Gadus callarias*, *Pseudopleuronectes americanus*
Habitat: Alimentary tract, mainly intestine
Incidence and abundance: Sculpin —June 17, 1947, 3 specimens from 1 fish
Cod —June 30, 1947, 1 specimen from 1 fish
Flounder—June 11, 1947, 2 specimens from 1 fish
June 28, 1947, 4 specimens from 1 fish

Discussion

The specimens from the three hosts agreed with the descriptions of *Podocotyle atomon* of Odhner (25), Manter (21), Linton (16), and Miller (23) in most fundamental respects and they were therefore referred to this species. The only characteristic causing some difficulty was the position of the vitellaria. All descriptions of *P. atomon* mention the fact that the vitellaria run laterally in an unbroken line and do not come together between the testes. Most of the material that was examined, however, although agreeing in the first point, does have vitellaria filling the intertesticular space to a varying extent.

This fact has been observed before by various authors. Linton (16) stated: "In some cases there is little or no interval between the testes, in others there is an interval into which follicles of vitellaria are inserted". Manter (21) also mentioned a case where the vitellaria approach each other and he considered the oesophagus length to be the best characteristic. Park (26) found that the distribution of the vitellaria varies widely within a given species.

Dawes (6) emphasized the fact that *Podocotyle atomon* is a very variable species and voiced the opinion that "several species which occur outside Europe will certainly prove to be invalid, and the same might be said of some European forms".

During the present investigation, it was observed, while trying to orientate a specimen of *Podocotyle atomon* from *Pseudopleuronectes americanus* between the slide and the cover slip under the microscope, that a slight pressure brought to bear accidentally on the trematode caused the yolk follicles to be pushed into the intertesticular space. This is interesting in connection with Nicoll's statement (24) "that artefacts produced by inappropriate methods of fixation modify specimens so that they answer to the descriptions of other species".

Miller (23), in re-examining Stafford's slide material, created the new species *Podocotyle staffordi* from *Gasterosteus aculeatus* (stickleback). One of the distinctive characteristics of this new species was said to be the fact that "*P. staffordi* has the vitellaria filling the intertesticular space but, unlike *P. olssoni*, the vitellaria are not discontinued laterally in the region of the testes".

The opportunity was afforded to examine that same slide material and in one specimen, labelled *Podocotyle atomon* from *Acanthocottus scorpius*, the yolk glands came together between the testes and were continuous laterally.

From the table (Table II) comparing the measurements of Miller's *P. atomon* and *P. staffordi* with the *P. atomon* collected during the present investigation, it would appear that there is a general overlapping of characteristics and that all the specimens belong to the highly variable species, *P. atomon.*

TABLE II

	P. atomon, Miller's material	*P. staffordi,* Miller's material	*P. atomon,* present material
Total length	1.45 to 3.81 mm.	1.07 to 1.57 mm.	4.33 mm.
Relation of length to width	1 : 4, 1 : 3	1 : 5, 1 : 6	1 : 6
Oesophagus length in relation to pharynx length	As long or longer than pharynx	Somewhat longer than pharynx	Longer than pharynx
Testes	½ width of body	½ width of body	A little less than ½ width of body
Vitellaria	Continuous laterally, do not fill intertesticular space	Continuous laterally, fill intertesticular space	Continuous laterally, fill intertesticular space

Podocotyle reflexa (Creplin, 1825)

Host: Microgadus tomcod

Habitat: Intestine

Abundance: June 11, 1947, 3 specimens from 1 fish

Discussion

The material at hand agreed in most points with Odhner's description, with testes occupying almost the whole body width and a relation of the length of the body to its width as 1 to 10.

However, the cirrus sac reached just halfway the distance from the acetabulum to the ovary and Odhner (25) mentioned that aspect as a characteristic of *P. olssoni.* Miller (23) found this same condition in a specimen from the hake.

Furthermore the oesophagus length of the present specimens equals that of the pharynx, another feature of *P. olssoni.*

The material was assigned to *P. reflexa* with some doubt, especially since the same fish contained specimens that were identified as *P. olssoni.* There is a possibility that differential degrees of contraction caused them to assume a misleading appearance.

Dawes (6) quoted Nicoll (24) as finding "that variability affects certain characters upon which Odhner depended for the determination of *P. reflexa* e.g. shape, flattening, relative position of the suckers", and also mentioned that *P. reflexa* and *P. olssoni* may be identical with *P. atomon.*

Podocotyle olssoni Odhner, 1905

Host: Microgadus tomcod

Habitat: Intestine

Abundance: June 11, 1947, 3 specimens from 1 fish

Discussion

The material was found in association with *P. reflexa* in a tomcod and there may be a possibility that it represents strongly contracted specimens of the latter species or that *P. reflexa* specimens belong really to *P. olssoni.* In the discussion of *P. reflexa,* the possibility of assigning *P. reflexa* and *P. olssoni* to *P. atomon* was also mentioned.

Steringophorus furciger (Olsson, 1868)

Host: Pseudopleuronectes americanus

Location: Alimentary tract

Abundance: June 28, 1947, 4 specimens from 1 fish

Discussion

The specimens were typical *Steringophorus furciger* according to the description of Linton (16), Miller (23), and Dawes (6).

Otodistomum cestoides (Beneden, 1870)

Host: Raja scabrata

Habitat: Intestine

Abundance: Aug. 29, 1947, 1 specimen from 4 fish

Discussion

The trematode was obviously immature so that neither the eggs nor the genital papillae could be measured and it was assigned to *Otodistomum cestoides* rather than *O. veliporum* purely on the basis of its distribution.

Van Cleave and Vaughn (34) maintained "that the American forms comprise a single highly variable species to which the name *Otodistomum cestoides* should be applied".

Dawes (6) felt that if the egg size of two of the subspecies of Dollfus varied to such an extent as was shown by Van Cleave and Vaughn, "then the remaining subspecies are invalidated" and "it seems logical to conclude that a single species of the genus *Otodistomum* infects cartilaginous fishes all over the world, and that it should be known not as *O. cestoides* but as *O. veliporum*.

Although this conclusion seems logical, the problem is still in an unsettled condition and the present species was, for the time being, identified as *O. cestoides*.

Brachyphallus crenatus (Rudolphi, 1802)

Hosts: Clupea harengus, Osmerus mordax, Salmo salar

Habitat: Stomach and remainder of digestive tract in the herring and smelt. One specimen was found on the gills of salmon. It may be noted here that trematodes frequently migrate from the alimentary tract to the gills in fish that have been dead for some time.

Incidence and abundance: Herring—July 28, 1947, 23 specimens from 11 fish
July 31, 1947, 19 specimens from 5 fish
Aug. 18, 1947, 6 specimens from 5 fish
Smelt —June 11, 1947, 1 specimen from 3 fish
June 20, 1947, 54 specimens from 1 fish
Salmon—June 30, 1947, 1 specimen from 1 fish

Discussion

The specimens met with in this investigation were typical *Brachyphallus crenatus* with lobed vitellaria and a well defined presomatic pit.

This species was first recorded from America by Lander (13), from *Osmerus mordax*, who described its anatomy in detail under the name *Hemiurus crenatus*.

The typical condition of the vitellaria of *Brachyphallus crenatus* was found in the present material, agreeing with the observations of Lloyd (17), Manter (21), and Linton (16) regarding the American forms of the species.

Hemiurus levinseni Odhner, 1905

Host: Gadus callarias

Habitat: Alimentary tract

Abundance: July 16, 1947, 6 specimens from 100 fish
July 24, 1947, 22 specimens from 31 fish
Aug. 3, 1947, 8 specimens from 12 fish
Aug. 8, 1947, 30 specimens from 21 fish
Aug. 13, 1947, 5 specimens from 100 fish

Discussion

The present material was determined as belonging to *H. Levinseni* by consulting Odhner's (25) description.

Lecithaster confusus Odhner, 1905

Host: Osmerus mordax

Habitat: Stomach

Abundance: June 20, 1947, 1 specimen from 1 fish

Discussion

Lühe's (18) and Linton's (16) descriptions were used in the identification. The main problem was to differentiate *Lecithaster confusus* from *L. gibbosus.*

Lühe (18) used the position of the seminal vesicle in relation to the ventral sucker as a characteristic, stating that it extends behind the ventral sucker in *L. confusus* but not *L. gibbosus.* He also mentioned the shape of the ovary as being rounded in *L. gibbosus* and longish in *L. confusus.*

Neither the seminal vesicle nor the ovary could be distinguished clearly in the single available specimen. However, the lobes of the vitellaria were short and scarcely as long as broad, agreeing with Lühe's description. Furthermore, the measurements placed the present material in the species *L. confusus.*

Derogenes varicus (Müller, 1784)

Hosts: Gadus callarias, Osmerus mordax, Salmo salar

Habitat: Gills, stomach. As was mentioned before in considering *Brachyphallus crenatus*, the location of a digenetic trematode on the gills of a fish is probably due to the migration of the parasite from the alimentary tract following the death of the host.

Incidence and abundance: Cod —July 24, 1947, 11 specimens from 31 fish
Aug. 4, 1947, 15 specimens from 12 fish
Aug. 8, 1947, 37 specimens from 21 fish
Aug. 11, 1947, 13 specimens from 10 fish
Aug. 13, 1947, 18 specimens from 100 fish
Smelt —June 30, 1947, 36 specimens from 1 fish
Salmon—July 3, 1947, 18 specimens from 1 fish

Discussion

This was by far the most abundant species of trematode encountered, occurring in large numbers in the parasitized host specimens, often giving the stomach lining a yellow appearance by their presence. Manter (21) mentioned that it occurred in many host species but in small numbers, but this does not seem to be the case in the present material. The incidence given above for this case is misleading since only representative samples were collected. The specimens agreed with Odhner's (25) and Dawes (6) descriptions.

B. Cestoda

Phyllobothrium dagnalli Southwell, 1927

Hosts: Raja scabrata, Raja stabuliforis

Habitat: Stomach, spiral valve, intestine

Incidence and abundance: Prickly skate—Aug. 25, 1947, 2 specimens from 4 fish
Aug. 29, 1947, 4 specimens from 3 fish
Aug. 30, 1947, 1 specimen from 5 fish
Barndoor skate—Aug. 27, 1947, 3 specimens from 1 fish

Discussion

The material agreed fully with the description of *P. dagnalli* of Southwell (32) from Ceylon.

Anthobothrium cornucopia van Beneden, 1850

Host: Raja scabrata

Habitat: Stomach, spiral valve, intestine

Abundance: Aug. 25, 1947, 2 specimens from 4 fish
Aug. 29, 1947, 3 specimens from 3 fish
Aug. 30, 1947, 24 specimens from 5 fish

Discussion

According to the keys of Joyeux and Baer (9), the material appeared to belong to the species *Anthobothrium cornucopia.* However, the description in "Cestodes de France" is very short. Southwell (32) considered the genus *Anthobothrium* to be included in the genus *Phyllobothrium* but did not mention the type species *A. cornucopia.*

The drawing of Fuhrmann (8) of a scolex of *A. cornucopia* from *Galeus canis* seems to show a myzorhynchus and no loculi as in the case of the present specimen. Fuhrmann gave the length of the cestode as 20 cm. but the material at hand corresponds rather with Joyeux and Baer's measurements, 10–252 mm.

Scyphophyllideum giganteum (van Beneden, 1859)

Host: Raja scabrata

Habitat: Intestine

Abundance: Aug. 29, 1947, 1 specimen from 3 fish
Aug. 30, 1947, 2 specimens from 5 fish

Discussion

These specimens belonging to the species *Scyphophyllideum giganteum* differ from all other Phyllobothriidae in having enormous globular bothridia, which have the appearance of suckers such as are found in the Cyclophyllidea. They were identified by consulting Woodland (40) and Joyeux and Baer (9).

Echinobothrium raji n. sp.

(Figs. 1 and 2)

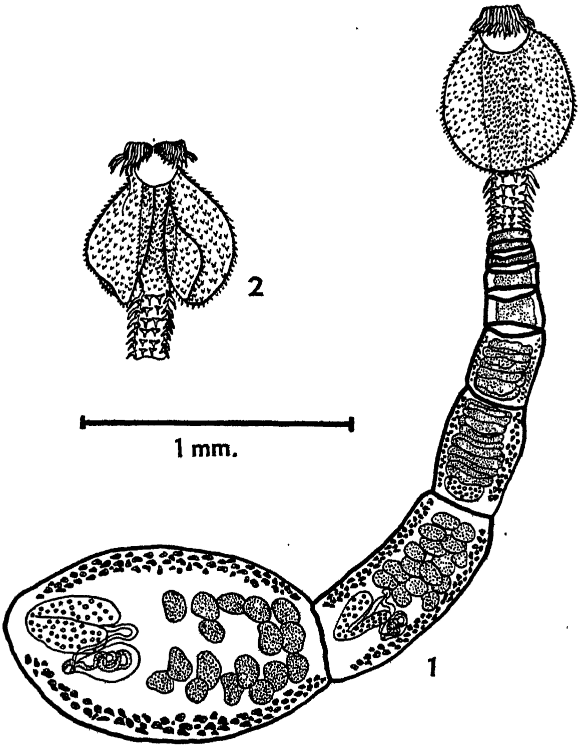


FIG. 1. *Echinobothrium raji* n. sp. entire worm, ventral view.

Fig. 2. *Echinobothrium raji* n. sp. scolex, lateral view.

Host: Raja scabrata
Habitat: Stomach, spiral valve
Abundance: Aug. 25, 1947, 21 specimens from 4 fish
Aug. 29, 1947, 1 specimen from 3 fish

Description: The length of the cestodes with seven proglottids is about 3 mm. Often eight or nine proglottids are present, depending at what stage the ripe segments detach themselves. The scolex measured to the base of the cephalic peduncle ("Kopfstiel") is 0.850 mm. long and the head, including the rostrum and "neck" but not the armed portion ("Kopfstiel") measures 0.675 mm. in length. The "neck" has a width of 0.195 mm. and a length of 0.480 mm. The rostrum with a diameter of 0.150 mm. bears, on either side, groups of 30 to 32 hooks arranged in two rows. On each side of the big hooks there are six small hooks. Each large hook is 0.113–0.120 mm. long and the small hooks decrease in size going toward the edge of the cluster. There are no spines on the base of the rostrum, but some small ones are found on the "neck", which is overhung by two lappets or pseudobothridia. The latter are also covered by minute spines and measure 0.605 mm. in length and 0.540 mm. in width. The cephalic peduncle bears eight longitudinal rows of eight hooks each. These hooks are directed backwards and diminish in length going toward the posterior end. The longest of these hooks are 0.024 mm. long and they are all anchored to the body by a triradiate base. Following the cephalic peduncle, there are three to four immature proglottids wider than long, but increasing in length toward the posterior end of the worm. The first few immature segments show hardly any structure but in the last ones the genital organs are seen. These mature proglottids increase in size becoming longer than wide and the last attached one measures approximately 1.2 mm. in length and 0.525 mm. in width. The cirrus is covered by spines and situated in a cirrus sac opening in the posterior third of the segment. The vagina opens next to the cirrus sac and leads to the U-shaped ovary situated in the posterior end of the segment. There are about 16 testes, which are square in the first proglottids and gradually assume a round shape. The yolk follicles fill the lateral space and the eggs are 0.013 mm. by 0.018 mm., bluntly rounded at one end and pointed at the other.

Discussion

Of the four species described briefly by Joyeux and Baer (9) and in greater detail by Pintner (27), the present species differs from *E. affine* Diesing in size, in the greater number of rostral hooks, and the smaller number of hooks on the cephalic peduncle as well as in the greater number of testes; from *E. brachysoma* Pintner in size, in the number of rostral hooks, hooks on the cephalic peduncle, and number of testes, as well as in the arrangement of the eggs; and from *E. typus* van Beneden in the number of rostral hooks, as well as the number of hooks on the cephalic peduncle and the number of testes.

E. musteli Pintner resembles the present species more closely than the other three, but it differs in having small spines on the base of the rostrum, in the larger number of hooks on the cephalic peduncle, and in the larger number of testes. But *E. raji* and *E. musteli* resemble each other in having nearly the same number of hooks on the rostrum and in the relatively great length of the scolex compared to the rest of the body.

Southwell (32) cited van Beneden's description of *E. typus* and concluded that his own *E. boisi* Southwell, 1911, was synonymous with *E. typus*. He further described *E. affine* and a curious species *E. rhinoptera* Shipley and Hornell, 1906, of which he said: "The two points in which this cestode differs from the other members of the genus, e.g. *E. affine*, *E. typus*, *E. brachysoma* and *E. musteli* are the complete absence of any spines on the head and the presence of a naked region or "neck" between the head and the armed region of the body" but Southwell added that the head hooks were probably lost. *E. longicolle* Southwell, 1925, described by its discoverer (32) has 180 hooks in each anteroposterior row of the "Kopfstiel".

Tentacularia coryphaenae Bosc. 1802

Host: Salmo salar

Habitat: Body cavity

Abundance: June 30, 1947, 2 specimens from 1 fish

Discussion

The two specimens, at the postlarval stage, were found moving around freely in the body cavity of a salmon. They were off-white and looked like portions of the caeca.

Dollfus (7) cited the opinion of Leuckart, von Siebold, and Pintner in favor of identifying *T. coryphaenae* with *T. quadrirostris*, and stated: "Ce nom (*T. coryphaenae*) a été établi pour la post-larve (ou larve) trouvée par Bosc chez *Coryphaena*; il est postérieur au nom spécifique donné par Goeze à la post-larve (ou larve) trouvée par Goeze chez *Salmo*; j'estime à peu près certain que *quadrirostris* et *coryphaenae* sont la même espèce, mais je n'ai pu me procurer, pour vérification, de spécimens trouvés chez des *Salmo*; je conserve donc, provisoirement, le nom spécifique *coryphaenae*; lorsqu'il sera formellement établi qu'il n'existe aucune différence spécifique, il faudra abandonner définitivement *coryphaenae* au profit de *quadrirostris*."

The present specimens from *Salmo* seem to be fully in agreement with Dollfus' description of *T. coryphaenae*, from *Coryphaena* giving support to the establishment of the name *T. quadrirostris*, which has priority. To be certain however, one would have to be able to compare actual specimens from *Coryphaena* and *Salmo*, especially in respect to the size and arrangement of proboscis hooks.

Grillotia sp. (W. Kahl, 1937)

Host: Raja scabrata

Habitat: Spiral valve, intestine

Abundance: Aug. 25, 1947, 10 specimens from 4 fish
Aug. 30, 1947, 2 specimens from 5 fish

Discussion

The material has all the characteristics of *Grillotia* sp. including the median insertion of the retractor in the proboscis sheath, and the homogeneous armature of the proboscis. The proboscis hooks also correspond to the detailed drawing of Dollfus (7).

Abothrium gadi van Beneden, 1871

Host: Gadus callarias

Habitat: Pyloric caeca, intestine

Abundance: June 25, 1947, 1 specimen from 34 fish
June 30, 1947, 1 specimen from 1 fish
July 7, 1947, 3 specimens from 15 fish
July 16, 1947, 1 specimen from 100 fish
July 24, 1947, 4 specimens from 31 fish

Discussion

The specimens from cod were determined as *Abothrium gadi* according to Joyeux and Baer (9).

Eubothrium crassum (Bloch, 1779)

Host: Salmo salar

Habitat: Pyloric caeca, intestine

Abundance: June 18, 1947, 1 specimen from 1 fish
June 20, 1947, 1 specimen from 1 fish
June 30, 1947, 3 specimens from 1 fish
July 3, 1947, 2 specimens from 1 fish

Discussion

The material was found to be identical with *Eubothrium crassum* as discussed by Wardle (35, 36), Kuitunen-Ekbaum (12), and Joyeux and Baer (9). Cooper (4) considered the same species under the name *Abothrium crassum.*

Diplocotyle olrikii Krabbe, 1874

Host: Pseudopleuronectes americanus

Habitat: Intestine

Abundance: June 28, 1947, 17 specimens from 1 fish

Discussion

The present material was referred to the species *Diplocotyle olrikii* by consulting Wardle's (35, 36) description. Cooper (4, 5) described similar specimens under the name *Bothrimonus intermedius.* Cooper, 1917 and Wardle's (35, 36) comment on this point was: "The species appears to be essentially a parasite of salmonoid fishes. The material described by Schneider, by Nybelin, and by Cooper was taken from pleuronectid fishes, which are probably abnormal hosts. The creation of separate species for such abnormally located specimens of *D. olrikii* purely on the basis of differences from Krabbe's description, seems to me unjustifiable".

In the specimens examined in this study, the two bothridial apertures are always distinctly separate.

C. ACANTHOCEPHALA

Echinorhynchus gadi Miller, 1776

Hosts: Gadus callarias, Microgadus tomcod

Habitat: Intestine

Incidence and abundance: Cod —June 20, 1947, 1 specimen from 1 fish
June 30, 1947, 63 specimens from 1 fish
July 7, 1947, 44 specimens from 15 fish
July 16, 1947, 25 specimens from 100 fish
July 21, 1947, 13 specimens from 24 fish
July 24, 1947, 38 specimens from 31 fish
Aug. 4, 1947, 17 specimens from 12 fish
Aug. 5, 1947, 16 specimens from 9 fish
Aug. 8, 1947, 18 specimens from 21 fish
Aug. 11, 1947, 6 specimens from 10 fish
Tomcod—June 11, 1947, 44 specimens from 1 fish

Discussion

Lühe's (19) and Markowski's (22) descriptions and drawings referred the present material to the species *Echinorhynchus gadi.*

D. NEMATODA

Porrocaecum sp. larvae

Host: Gadus callarias

Habitat: Muscles, body cavity

Abundance: The abundance of this and the following nematode species is not given since it would only be misleading. Representative samples only were collected since the total amount of parasites present was too great.

Discussion

All the larval forms with an intestinal caecum and a ventriculus were determined as belonging to the genus *Porrocaecum.* Some of the smaller larvae without caeca may also have belonged to this genus since according to Baylis (1): "The caecum of this type of larva (*Porrocaecum*) is only developed gradually during its sojourn in the fish host, and the young larvae, before its development, cannot be distinguished with any certainty from those of *Anisakis*".

Baylis (1) and Punt (28) both questioned Kahl's (11) and Wülker's (41) ability to identify specifically larval stages of ascarids in marine fishes and gave excellent reasons to disregard Kahl's and Wülker's identification.

Contracaecum aduncum (Rudolphi, 1802)

Hosts: Gadus callarias, Tautogolabrus adspersus, Microgadus tomcod, Salmo salar, Acanthocottus scorpius, Pseudopleuronectes americanus, Scomber scombrus

Habitat: Stomach, intestine, body cavity, muscles

Discussion

Adults as well as larvae of *Contracaecum* were found. The larvae could not be identified specifically but the possession of an intestinal as well as an oesophageal caecum clearly placed them in the genus *Contracaecum*; and it seems very probable that they belong to the species *C. aduncum* since they were found in the same host specimens as the adults of that species. The adults were determined as being *C. aduncum* by referring to the illustrated descriptions of Kahl (10), Punt (28), and Markowski (22).

Kahl (10), as well as most authors, recorded this nematode under the name *C. clavatum* but Punt (28) in reviewing the literature remarked that no author after *A. Schneider* saw all the species of *Contracaecum* one beside the other and, as a consequence, many synonymous names appeared. Punt also stated that the specific diagnosis of these species is based on the structure of the lips, but that a detailed comparative study of the literature as well as of actual material did not reveal any significant differences between many forms. He drew up a list of synonyms, including *C. clavatum*, to be replaced by the name *C. aduncum*, which has priority. The arguments of Punt seem very convincing and the present material was, therefore, determined to belong to the species *Contracaecum aduncum.*

Eustoma rotundatum (Rudolphi, 1819)

Host: Raja erinacea

Habitat: Stomach, spiral valve

Abundance: Aug. 15, 1947, 11 specimens from 2 fish

Discussion

The present specimens were identified according to the description and drawings of Punt (28) with which they agreed in all respects. Linton (15) gave an illustration of the same species under the name *Ascaris rotundatus.*

Anisakis sp. larvae

Hosts: Gadus callarias, Clupea harengus, Microgadus tomcod, Salmo salar, Osmerus mordax, Urophycis chuss, Scomber scombrus

Habitat: Stomach, intestine, body cavity

Discussion

The larval forms, possessing a ventriculus but lacking caeca, were referred to this genus. Some of these may really represent young forms of *Porrocaecum* and this question has been discussed when considering the larvae of *Porrocaecum*.

(3) ANTHROPODS

A. COPEPODA

Caligus curtus Müller

Host: Gadus callarias

Habitat: Outside surface

Abundance: Almost all cod examined had these copepods on their skin. The total number per fish could not be determined accurately since the cod had usually been stored for some time in the hold of a ship and been handled extensively before examination, causing many external parasites to become detached. The average number per fish seemed to be 5 to 10 parasites.

Discussion

Both the males and the females were encountered and corresponded to Wilson's (39) description.

Lepeoptheirus salmonis (Kröyer)

Host: Salmo salar

Habitat: External surface

Abundance: On every salmon examined. Many were undoubtedly rubbed off during handling of the fish and therefore the total number per fish could not be recorded.

Discussion

T. and A. Scott's (29, 30) account of the species was consulted and led to its diagnosis. Both males and females were found.

Lernaeocera branchialis (Linnaeus)

Host: Gadus callarias

Habitat: Gill arches

Abundance: June 25, 1947, 1 specimen from 34 fish
June 30, 1947, 1 specimen from 1 fish
July 3, 1947, 1 specimen from 10 fish

Discussion

All three specimens that were found were females. Wilson (39) and T. and A. Scott (29, 30) gave excellent descriptions and illustrations of this species.

Charopinus cameroni n. sp.

(Figs. 3 and 4)

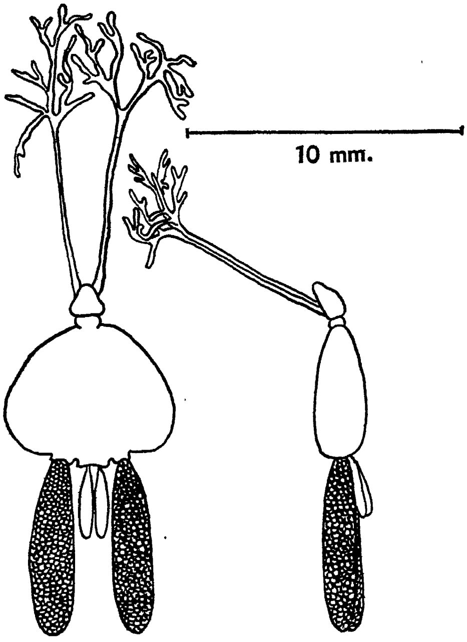


FIG. 3. *Charopinus cameroni* n. sp. dorsal view.

FIG. 4. *Charopinus cameroni* n. sp. lateral view.

Host: Raja scabrata

Habitat: External surface

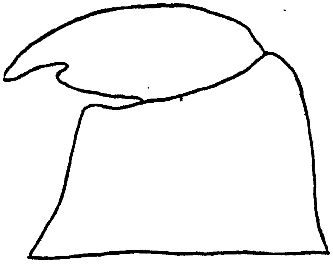
Abundance: July 16, 1947, 2 specimens from 1 fish
Aug. 25, 1947, 2 specimens from 4 fish
Aug. 30, 1947, 5 specimens from 5 fish

Description

Female

The head is almost triangular in dorsal view and is inclined forwards or ventrally at a 45° angle. This is followed by a short neck, narrower than the base of the head and consisting of a single segment. The trunk is relatively plump, much wider than the head and almost oval, with the longer axis directed sideways. The posterior end of the trunk is emarginated and bears the egg sacs and posterior processes. The egg sacs are quite plump and long, being twice the length of the trunk. The posterior processes are situated dorsal to the egg sacs.

(Figs. 5, 6, 7, 8 and 9)



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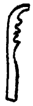

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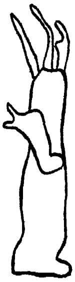

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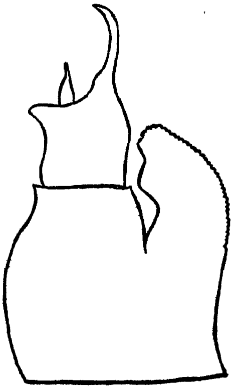

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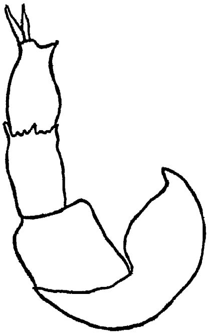

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FIG. 5. *Charopinus cameroni* n. sp. maxilliped.

FIG. 6. *Charopinus cameroni* n. sp. mandible.

FIG 7. *Charopinus cameroni* n. sp. first maxilla.

FIG. 8. *Charopinus cameroni* n. sp. second antenna.

FIG. 9. *Charopinus cameroni* n. sp. first antenna.

The first antenna is four-jointed and bears two terminal spines as well as several spines at the joint between the last and penultimate segment. The second antenna is almost chelate with a curved exopod, having a dentate margin and a jointed endopod. The endopod has two terminal spines. The first maxilla is tipped with three large setae and bears a bifid palp. The second maxillae are somewhat longer than the body, excluding the egg sacs. There is no bulla and the ends of each maxilla ramify throughout the host tissue. The mandibles are narrow and long and bear a small number of coarse teeth. The maxillipeds are very stout and chelate, with a curved second joint articulating at right angles to the first joint.

No males were found.

Measurements: Average female specimen

Total length	2 cm. (including egg sacs)
Cephalothorax	2 mm. length
	3 mm. width (maximum)
Neck	0.75 mm. length
Trunk	5 mm. length
	7 mm. width
Posterior processes	2 mm. length
	0.7 mm. width
Egg sacs	1 cm. long
First antenna	0.180 mm. long, 0.120 mm. wide at base
	0.040 mm. wide at tip
Second antenna	0.135 mm. wide at base
	0.125 mm. long
First maxilla	0.150 mm. long, 0.037 mm. wide
Second maxilla	8 mm. long
Maxilliped, 1st joint	0.195 mm. wide
	0.170 mm. long
2nd joint	0.150 mm. long
	0.075 wide
Mandible	0.060 mm. long
	0.0075 mm. wide

Discussion

The present species belongs to the genus *Charopinus* Kröyer as defined by Wilson (38), the most striking feature being the lack of a bulla, a character which differentiates it, among others, from the genus *Lernaeopodina.* Wilson described the cephalothorax as being bent dorsally but T. and A. Scott (29, 30) show *Charopinus* (*Lernaeopoda*) *cluthae* and *Charopinus ramosus* with the cephalothorax bent forwards so that the present species is not to be excluded from the genus on that basis.

The closest resemblance to the present material, within the genus *Charopinus*, is shown by *Charopinus ramosus* as described and figured by T. and A. Scott (29, 30). This species has the cephalothorax projecting forwards and the mouth parts exhibit a great similarity to the specimens at hand. The main difference of the mouth parts lies in the shape of the first maxilliped, which has a long and slender second joint. The first maxilliped of *C. dalmanni* (30) has a shape identical to that of *C. cameroni.* The mandible of *C. ramosus* also differs from the new species in being obliquely truncate at the tip. The mouth parts of *C. cluthae* (30) are also similar but this species possesses a bulla.

The body shape and the proportions as well as the configuration of the second maxillae differentiate the known species of *Charopinus* from the present species.

The material is therefore assigned to the new species, *Charopinus cameroni*, in honor of Dr. T. W. M. Cameron.

Bere (2) in her description of *Lernaeopodina longimana* from *Raja scabrata* from the Bay of Fundy mentioned a strange specimen, differing from the others in possessing second maxillae without a bulla and with dichotomous ramefying branches. The first maxillae of this specimen were "tipped with three setae instead of two", like *C. cameroni*, but "the palp, instead of having a bifid appearance ended bluntly and bore three small spines". It is possible that this was a specimen of *Charopinus cameroni.*

Clavella uncinata (Müller)

Host: Gadus callarias

Habitat: Gills, buccal cavity, anal region

Abundance: These copepods are situated in more sheltered places and are more securely attached than *Caligus or Lepeoptheirus* and the number observed on each fish probably gives the true abundance of infection. The number cited refers to the female specimens. One or two males were usually found attached to each female.

June 24, 1947, 2 specimens from 1 fish
June 25, 1947, 13 specimens from 34 fish
July 3, 1947, 5 specimens from 10 fish
July 7, 1947, 18 specimens from 15 fish
July 11, 1947, 4 specimens from 70 fish
July 21, 1947, 42 specimens from 21 fish
Aug. 4, 1947, 14 specimens from 12 fish
Aug. 5, 1947, 2 specimens from 9 fish
Aug. 8, 1947, 28 specimens from 21 fish
Aug. 11, 1947, 4 specimens from 10 fish
Aug. 13, 1947, 9 specimens from 100 fish

Discussion

Wilson (39) and T. and A. Scott (29, 30) reported the species *Clavella uncinata* from the gills of gadoids and the present material seems to agree with their descriptions.

Leigh-Sharpe (14) in his revision of the British species of *Clavella* stated: "The members of the genus are pre-eminently parasitic on gadoid fishes, each species of *Gadus* is accompanied by a characteristic species of *Clavella.* Where two or more species of the latter occur in the same host, each has a definite location, thus with

Gadus morrhua ⟨ *C. sciatherica* occurs in buccal cavity, pharynx.
C. iadda occurs on the skin, fin, tail, anus.

One of the most decisive characters serving for identification is the bulla. This varies according to the species, (*a*) in shape being spherical, ovate, clavate, or otherwise and (*b*) in respect to its channelings. In *C. iadda* each channel divides into four. In *C. sciatherica* the two channels unite to form a single channel, which afterwards forks, each branch dividing into four". He also reported that the bulla of the latter is spherical and that of the former ovate, and he gave drawings to illustrate these points. Leigh-Sharpe doubted the existence of the species *Clavella uncinata.*

In the present investigation, the material from the gills and buccal cavity was not kept separate from that of the anal region because it was not realized, at the time of collection, that there was a possibility of two species being present. But although numerous specimens were collected from the latter region, a thorough examination of each bulla revealed only one that could correspond to the description of *C. iadda.*

Furthermore, Bere (1) and especially Wilson (39) reported only the one species *Clavella uncinata* from North American cod, although they must have been aware of Leigh-Sharpe's previous publication.

The present material is therefore assigned to the species *Clavella uncinata.*

Acknowledgments

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THE DISTRIBUTION OF RADIOACTIVE ARSENIC IN THE ORGANS OF POISONED INSECT LARVAE[1]

By F. O. Morrison[2] and W. F. Oliver[3]

Abstract

Radioactive arsenic trioxide, containing As^{76} injected in solution into the haemolymph of last instar *Tenebrio molitor* L. larvae, distributed itself throughout the haemolymph, body wall, fore-, mid-, and hindgut, and Malpighian tubules within one hour and showed little change in distribution after as much as 20 hr. even though most larvae remained alive. When injected into the gut it showed little sign of escaping. Fifty instar *Phlegethontius quinquemaculata* Haw. larvae fed the dry active arsenic trioxide absorbed only very small amounts. The largest portion remained in the gut if the specimen died or was eliminated within 20 hr. if the specimen lived. As little as 10^{-11} gm. arsenic was detectable in the digested insect organs. The use of a dipping counter on aliquots of the acid tissue digest provides a simple and accurate means of counting samples and is to be recommended in studies of this nature.

Introduction

It appears that the quantitative determination of radioactive insecticides in the individual organs of poisoned insects may prove a very delicate technique for following a toxin to the seat of activity and thus throw light on the fundamental action involved (1, 2, 3). With this in mind the authors undertook to investigate the possibilities of radioactive tracer work in the field of insect toxicology. Arsenic was chosen as the toxin because of its availability and nonvolatility.

Materials and Procedure

Arsenical Formulation

Active arsenic trioxide containing As^{76} was obtained from the National Research Council Laboratories at Chalk River. It was formed by As^{75} (n, γ) As^{76} reaction as indicated by the determined half-life of 26.77 hr. (Compare the value 26.8 hr. given in Seaborg's table (6).) The active insecticidal solution was prepared, on each occasion, as follows: 0.104 gm. of active arsenic trioxide (specific activity on arrival: 8 mc. per gm.) was added to 75 ml. of distilled water; 0.45 gm. of sodium carbonate and three drops of 40% sodium hydroxide solution were added to ensure solution of the arsenic trioxide; 1.981 gm. of sodium chloride was dissolved in this to render the solution approximately isotonic with the body fluids of the mealworm (5); and finally distilled water was added to make up to 104 ml.

Holder for Mealworm Larvae

A holder for mealworm larvae was constructed from two pieces of glass tubing of suitable bore, plugged at one end and held by pieces of rubber

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stoppers, fitted into grooves in a firm base. This was mounted under a low power binocular microscope.

Injection Apparatus

A No. 22 hypodermic needle was attached to a tuberculin syringe clamped firmly to a retort stand and so adjusted that a slight movement allowed the needle to penetrate the firmly held specimen dorsolaterally through the intersegmental membrane between the second and third abdominal segments. A hand lens was used to read the graduations on the syringe.

Digestion Apparatus

Standard micro-Kjeldahl flasks and still smaller flasks blown from pyrex tubing were used for the digestion of the organs.

Counter and Auxiliary Equipment

A thin glass walled dipping counter (Distillation Products Incorporated) with a quenching circuit was used. Aliquots of the digest were placed in glass tubes with internal diameter 3 mm. greater than the diameter of the counter. These were placed beneath the counter and raised by a rack and pinion. Thus the same relative position of sample and counter was easily reproduced each time. Samples were counted for sufficient time to give no more than 2% error.

Test Animals

Last instar mealworm, *Tenebrio molitor* L., larvae (Coleoptera; Tenebrionidae) weighing an average of 0.2191 gm. each, reared on whole wheat flour, and last instar northern tobacco hornworm, *Phlegethontius quinquemaculata* Haw., larvae (Lepidoptera: Sphingidae) supplied by Mr. C. J. S. Fox, Entomologist in Charge, Dominion Entomological Laboratory, Chatham, Ont., were used as test animals.

Experimental Procedure

1. Fifteen last instar mealworm larvae were dissected and the organs pooled and weighed wet in weighing bottles. Approximately 20% of the live weight was lost by desiccation during dissection.

2. Last instar mealworm larvae were injected with 0.01 ml. of radioactive arsenic trioxide solution (0.00001 gm. arsenic trioxide) and were retained for varying intervals before dissection. Several similarly injected larvae were placed on Eastman X-ray films to secure radioautographs.

After the desired interval larvae were dissected and separated into foregut, midgut, hindgut, Malpighian tubules, body contents, and body wall. These portions were then digested in small amounts of hot nitric and sulphuric acid* until the solution was clear. While the activity varied from 0.08 μc. to 0.003 μc. per injection it was possible to work with one larva at a time, but later the parts of from 2 to 10 larvae were grouped for digestion.

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* *This is the method of digestion used when determining arsenic in organic matter by the Gutzeit or bromate methods. The condensed vapors from the digestion of insect tissues containing larger amounts of nonradioactive arsenic trioxide showed no trace of arsenic by the former test.*

Attempts to precipitate the arsenic from the digest, filter it off, and count the activity of the dry precipitate proved subject to errors that made the reproduction of results very difficult. In the work reported here a dipping counter was used, the acid digest being diluted to 25 ml. at room temperature, and two aliquots of 4 ml. each pipetted out and "counted".

3. Last instar northern tobacco hornworm larvae were fed unmeasured amounts of the dry active arsenic trioxide by "buttering" it onto a tomato leaf. No difficulty was experienced in getting the larvae to consume the treated leaf area. These larvae were then kept on tomato leaves and later dissected. Digests of organs, faecal pellets, and regurgitated material were "counted" for radioactivity.

Results

1. Table I gives the results of mealworm larvae injection in terms of the percentage of total activity (arsenic) in each organ; Table II gives the same information per unit weight of organ. The latter values were determined by using the average wet weights of organs dissected from 15 last instar mealworm larvae. The weights were: body wall, 0.931 gm.; body contents, 0.0419 gm.; Malpighian tubules, 0.0034 gm.; foregut, 0.0013 gm.; and hindgut, 0.0029 gm. These figures total approximately 20% less than the average live weight of the 15 worms, the loss being accounted for by desiccation during the dissection operation. The loss is probably not uniform for all tissues as the liquid tissues

TABLE I

PERCENTAGE* OF TOTAL ARSENIC FOUND IN ORGANS OF LAST INSTAR *Tenebrio molitor* LARVAE DISSECTED AT VARYING INTERVALS AFTER 0.00001 GM. OF As_2O_3 HAD BEEN INJECTED INTO THE HAEMOLYMPH OF EACH LARVA**

Interval in hours (injection to dissection)	Number of larvae	Body wall	Body contents	Malpighian tubules	Foregut	Midgut	Hindgut
1	4	67	15	3	3	10	2
1.5	8	67	17	6		6	4
2.5	4	66	19	3	1	9	2
4	5	65	17	5	2	8	3
7	4	70	13	6	2	6	3
10	10	64	21	6	1	5	3
16	5	61	25	4	1	7	2
20	4	58	25	7	1	7	2

* *Expressed in terms of percentage of the sum of the amounts found in the organs.*

** *All counts have been corrected for background, counter variability, and decay, the last having been determined by actual counts on aliquots of a standard solution of known dilution of the arsenic trioxide.*

TABLE II

Arsenic distribution in organs of last instar *Tenebrio molitor* larvae dissected at varying intervals after 0.00001 gm. of As_2O_3 had been injected into the haemolymph of each larva: expressed as percentage of arsenic per unit wet weight of organ

Interval in hours (injection to dissection)	Number of larvae	Body wall	Body contents	Malpighian tubules	Foregut	Midgut	Hindgut
1	4	13	7	14	33	17	11
1.5	8	16	9	36		12	27
2.5	4	17	11	18	15	19	19
4	5	13	8	27	23	7	22
7	4	13	5	33	24	9	16
10	10	15	11	18	23	10	23
16	5	14	13	22	23	14	14
20	4	13	12	43	10	12	10

would be expected to suffer the greater loss and it therefore seems questionable whether the weights given above should be corrected for this loss or not before being used to determine the toxin per unit weight. No correction was made.

2. Radioautographs of injected mealworm larvae gave either diffuse pictures of the entire body resulting from haemolymph injection, or a faint line indicating the position of the gut when the arsenic had been delivered into the alimentary canal by the injection needle. Since reproductions from such pictures lack detail they are not included here. (Cf. Norton and Hansberry (4).)

3. A fifth instar hornworm larva fed an unmeasured quantity of active arsenic was dissected after two hours: faecal pellets passed at one hour after feeding showed 0.1% of the total activity; those passed after two hours, 9.1%; the body wall, 13.1%; and the Malpighian tubules, 0.8%; while the gut and body contents showed 77.2%. As the gut was punctured during dissection the activities of the gut and body contents were grouped together. Some slight contamination of the body wall may have resulted from the puncture.

A second hornworm larva dissected 19 hr. after being fed was still alive and had to be anaesthetized prior to dissection. The arsenic distribution was: faecal pellets, 90.0%; body wall, 3.1%; Malpighian tubules, 0.7%; body contents, 1.6%; and gut, 4.0%.

A third hornworm larva dissected 24 hr. after feeding, but dead for some time, showed: faecal pellets, 0.2%; regurgitated material, 12.1%; body wall, 7.8%; Malpighian tubules, 0.4%; body contents, 2.0%; and gut, 69.4%.

Discussion

It would appear that arsenic trioxide injected into the haemolymph of mealworm larvae is by the end of one hour distributed through all the organs and little change occurs after that, even though the larvae remain alive (though somewhat inactivated) for many hours. What appeared to be a trend of adsorption from haemolymph to organs in earlier tests made on larvae held back from pupation by refrigeration at 34° F. was not borne out in the later tests on nonrefrigerated larvae reported here. This difference might have been due to physiological inactivation. Expressed as percentage of arsenic per unit weight of organ the distribution was nearly uniform in all organs measured. Curiously enough the body contents, including the haemolymph into which the injection had been made, gave the lowest activity reading per unit weight in spite of the fact that the largest part of the weight loss during dissection was likely to have taken place from this liquid tissue.

The results demonstrate clearly the high sensitivity of the technique. Even after the activity had dropped to less than 1/1000 of the original, less than 1% of the total injected into a larva was measurable; in other words, detection of quantities of the order of 10^{-11} gm. was possible.

Attempts to hold back the development of tobacco hornworm larvae, so that they would be available as and when desired, failed. The size and readiness with which these test animals ingest dry material, however, makes them very desirable. Then too, their larger size makes possible the use of dosages detectable even after the activity of the material has fallen off greatly. The indication that ingested arsenic trioxide was largely retained in the gut of these animals and rapidly eliminated in the faecal matter of those that survive is what might be expected from the insolubility of arsenic in this form.

Acknowledgments

In this first published paper on results obtained in this laboratory using the tracer technique in agricultural problems it is the desire of the authors and of the Macdonald College Tracer Committee to thank the National Research Council for the financial aid that made an equipped laboratory possible and assisted in getting various projects of this nature under way.

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SOME EFFECTS OF TEMPERATURE AND OXYGEN PRESSURE ON THE METABOLISM AND ACTIVITY OF THE SPECKLED TROUT, *SALVELINUS FONTINALIS*[1]

By J. M. Graham[2]

Abstract

Two levels of oxygen uptake were measured at temperatures from 5° C. to 24° C. on yearling speckled trout having a mean weight of 27 gm. These levels were the minimum resting rate in the diurnal cycle (standard rate) and the rate when induced to activity in a rotating chamber (active rate). The trout were thermally acclimated to the temperatures at which the experiments were performed. The standard rate of oxygen uptake increased with increasing temperatures up to the ultimate upper lethal temperature (25.3° C.). The active rate of oxygen uptake increased with increasing temperatures up to approximately 19° C. Above this temperature it fell again. The consequence of this difference in the response of the standard and active rates of oxygen rate to temperature is that the difference between these two levels increases rapidly up to a temperature of 16° C. and falls again thereafter. This is interpreted as indicating that the optimum temperature for the activity of the speckled trout is in the neighborhood of 16° C. The activity of speckled trout in relation to temperature was measured in terms of the maximum speed that they can maintain indefinitely. This speed was also found to be a maximum at a temperature of 16° to 20° C., at which region occurs the greatest difference between the standard and active rates of oxygen uptake.

The oxygen uptake of active speckled trout was measured over decreasing partial pressures of oxygen, and was found to become dependent on the oxygen pressure below a certain critical level. These critical pressures change with temperature from 100 mm. Hg at 5° C. to 150 mm. Hg at 24.5° C. Water containing less than 75% air saturation of oxygen reduces the activity of speckled trout at all temperatures. Above 20° C. fully saturated water is required to allow full scope to the activity of speckled trout. The minimum oxygen requirements for existence of the speckled trout at the standard metabolic level are estimated to vary from 30 mm. Hg at 5° C. to 79 mm. Hg at 24.5° C. It must be emphasized, however, that these values are conservative. The lethal levels of oxygen were measured for speckled trout and found to rise from 19 mm. Hg at 3.5° C. to 45 mm. Hg at 23° C.

Introduction

The speckled trout, *Salvelinus fontinalis* (Mitchill), is characteristically an inhabitant of cool well-aerated waters. Speckled trout have been found to frequent waters varying in mean summer temperature from 10.5° C. (8) to 21° C. (7). Minimal safe oxygen concentrations for speckled trout have been set by field workers at 4 to 5 p.p.m. (7, 9, 12, 26).

The temperature relations of speckled trout have been studied in further detail in the laboratory. Brett (3, 4) and Fry, Hart, and Walker (17) established the upper lethal level of temperature for the species. Rogers (28) described the relation of temperature to cruising speed of speckled trout. Their preferred temperature has been investigated by Fisher and Elson (13) and again by Elson (11) in connection with its relation to the temperature at which the fish respond maximally to an electrical stimulus.

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The purpose of the present work is to supplement the investigations listed so as to permit the definition of the scope for continuous activity that various combinations of oxygen pressure and temperature may present to the speckled trout. The method of attack was that used by Fry and Hart (16) for the goldfish. It included essentially the measurement of the standard and active levels of metabolism over a range of temperatures and oxygen pressures, and the determination of maximum steady activity at the various temperatures. To delimit further the environment suitable for speckled trout the lower lethal levels of oxygen were estimated from the metabolic data and also determined by direct experimentation.

Materials and Methods

The speckled trout used in all these experiments were yearlings obtained from provincial hatcheries. Their weights ranged from 17 to 65 gm. with a mean of 27 gm. Liver was fed to the fish every two days, the feeding times being arranged so that at least 24 hr. elapsed between them and the time the fish were used in experiments. In all cases the fish were maintained at the temperature at which the experiment was to be carried out for a period of some days or weeks prior to the experiment.

The standard metabolism of the trout as described here is the minimum metabolic level found in the 24 hr. cycle. It is considered an approximation of the basal level. As is commonly the procedure, the standard metabolism was measured by recording the oxygen intake of the resting fish. The method used is described by Fry and Hart (16). Each fish was enclosed in a 2 l. flask through which flowed water of constant temperature. This flow was stopped over certain periods and the oxygen intake estimated by taking the difference between the oxygen content of the water in the flask before and after the stoppage. To accommodate the rise in metabolic rate with temperature, the duration of the period during which circulation through the flask was cut off was decreased from three hours at 5° C. to one hour at 10° C. and over. Water was allowed to flush through the flasks for at least an hour between each test period.

Keys (22), Wells (29), and Black, Fry, and Scott (2), among others, have recorded that handling of fish raises their oxygen consumption well above the resting level, and that the excited condition may last for some time after the fish is not visibly active. For this reason the speckled trout were left undisturbed in the flasks at least 24 hr. before any water samples were taken. The fish were fed liver an hour or so before being placed in the flasks so that a fairly standard nutritional state was obtained.

The active respiratory rates were obtained by measuring the oxygen intake of fish swimming at a maximum steady rate in opposition to the motion of water in a rotating chamber. The chamber and the details of the method of following the oxygen intake of active fish are described by Fry and Hart (16). In these experiments the usual procedure was to place two trout in a measured amount of water (3 or 4 l.) and to withdraw water samples for oxygen analysis every 10 to 20 min. Since periodic tests for nitrite in the water proved negative, the unmodified Winkler technique for the measurement of dissolved oxygen was used in determining both active and standard metabolic rates.

During the measurement of the active rate, the rotation was held at a speed that kept the fish as active as possible; no particular record was kept of the swimming rates, however. To test whether or not this course of action was justified, the oxygen intake was measured of one fish, first as the chamber remained still and then as it rotated at 27 r.p.m. Fig. 1 illustrates the fall of oxygen in the chamber as the fish extracted it over a period of time. Somewhat surprisingly, there is no difference in rate of oxygen intake within the two experimental conditions. The handling of the fish as it was first put into the chamber was evidently sufficient stimulus to activity and eliminated the necessity of further agitating during the next half hour or so. In this respect the trout behaves much as the perch, sunfish, and bullhead whose excitabilities have been similarly tested (2).

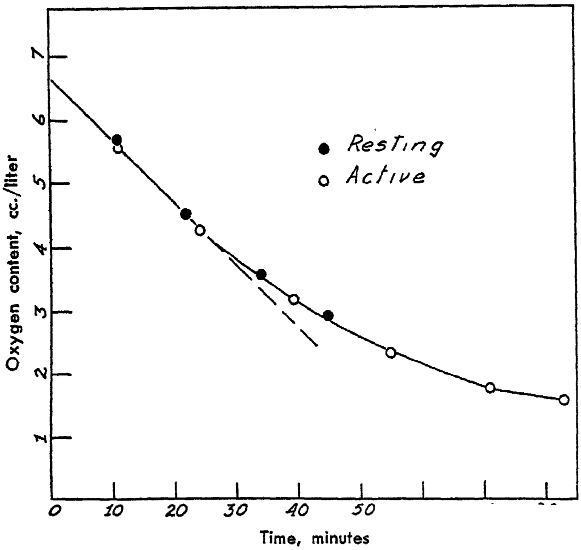

FIG. 1. *The extraction of oxygen from water in the annular chamber by a 62.3 gm. speckled trout acclimated to 14.5° C.*

Resting: the chamber at rest.

Active: the chamber rotating at 27 r.p.m.

The measurements at rest were taken first.

From the initial straight part of such curves as shown in Fig. 1, the maximum active respiratory rate was calculated. The amount of carbon dioxide that accumulated during these measurements of active metabolism does not appear to have significantly affected the rate of oxygen uptake. In 21 experiments, marble chips, which kept the carbon dioxide pressure to 4 mm. Hg and less, were placed in the bottom of the chamber. These experiments gave essentially the same result as when no chips were used. Without chips, carbon dioxide pressures of the order of 9 mm. were attained at the end of the longer runs.

Both the standard and active rates of oxygen uptake per unit weight were found to decrease with size, but the precise relationship has not been determined in the present instance. It must be realized, therefore, that the rates presented here, although reduced to rates per unit weight, hold good only for trout 20 to 65 gm. in weight.

Direct measurements of the lethal level of oxygen at four temperatures were made by subjecting the fish to running water in which the oxygen content had been reduced. The apparatus first used to measure lethal levels of oxygen consisted of five 2 l. Erlenmeyer flasks connected in series by rubber and glass tubing. Water of regulated temperature flowed through the series. As the water passed through successive flasks the fish therein lowered the oxygen content in such a way that a decreasing gradient of oxygen was set up between the first and last flasks. To remove the free carbon dioxide, the water flowed through a tube containing crushed marble as it passed from each flask to the next. The experiment recorded at 3.5° C. was done in this apparatus. It was found, however, that the oxygen content of the flasks fluctuated with the time of day. Apparently the greater level of external stimuli in the daytime (or an endogenous activity cycle) prompted the fish to increase their metabolic rates even though oxygen was withheld. For this reason the apparatus was discarded in favor of another, which included a fractionating column made of 1 liter flasks described elsewhere (15). Water introduced into the top of the column met nitrogen rising from an inlet at the bottom. As the water descended its oxygen content became progressively lowered so that it was possible to draw off water of a different constant oxygen level from each of the five flasks constituting the column. The oxygen content of the whole series could be raised or lowered by introducing the water at different distances up a four foot glass tube at the top of the column. For that purpose, four inlets were fused up the tube about 10 in. apart.

Five levels of oxygen lying about 0.5 cc. per liter apart could be obtained by use of this apparatus. Such a gradient was suitable to establish roughly the whereabouts of the lethal level. For a more exact determination, some of the flasks were replaced by test tubes fitted together and provided with outlets in the same manner as the flasks. There was a difference of about 0.1 cc. per liter of oxygen in water drawn from successive test tubes. To keep the gradient steady, especially when water above 15° C. was used, the temperature had to be maintained within 0.3° of the test temperature.

The trout were placed in Erlenmeyer flasks submerged in a constant temperature bath supplied by water from the Erlenmeyer overflows. To allow time for them to settle, the fish were fed and put into the flasks at least 12 hr. before the experiment was begun. During this preliminary period the

Erlenmeyers were flushed through with aerated water. At the same time the nitrogen was turned on in the equilibrator column so that the gradient was established and steady when needed. To start the experiment the rubber tubing supplying aerated water to the inlet of each Erlenmeyer was replaced by tubing from the outlets of the gradient column. In this way the oxygen levels in the Erlenmeyers could be changed without disturbing the fish. Each experiment was carried out over a minimum period of 24 hr.

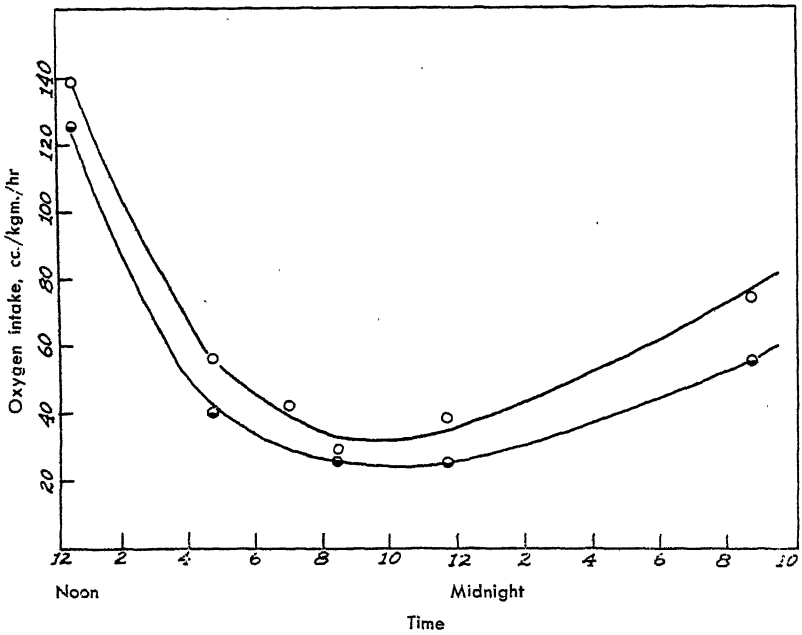

FIG. 2. *The relation of oxygen intake to time of day in two resting speckled trout acclimated to 5° C. Data of A. H. Lawrie (unpublished).*

Results

(a) The Effect of Temperature on the Standard Metabolic Rate

Although the room in which the standard metabolism experiments were done was kept reasonably quiet, and light was at a minimum, a diurnal fluctuation in the metabolic rate of the "resting" fish persisted. This behavior is common to many species of both fish and mammals (31) and was to be expected in the trout. An example of such a change of rate with time of day is displayed in Fig. 2, which is a plot of data obtained using trout acclimated to 5° C. Though the relation of this fluctuation to time of day would indicate a simple response to light intensities, there have been other experiments done with speckled trout (19) and mammals (21) in which it was found that the fluctuation persisted for some time even in continued darkness.

The lowest activity occurred at approximately 12 p.m. in experiments done over 24-hr. periods at 5°, 10°, and 15° C. Consequently, the respiratory rates were measured only at night for the last three acclimation temperatures, starting at 11 p.m. and concluding at 7 a.m. The lowest point on each plot of respiratory rate against time of night was taken as the standard metabolic rate.

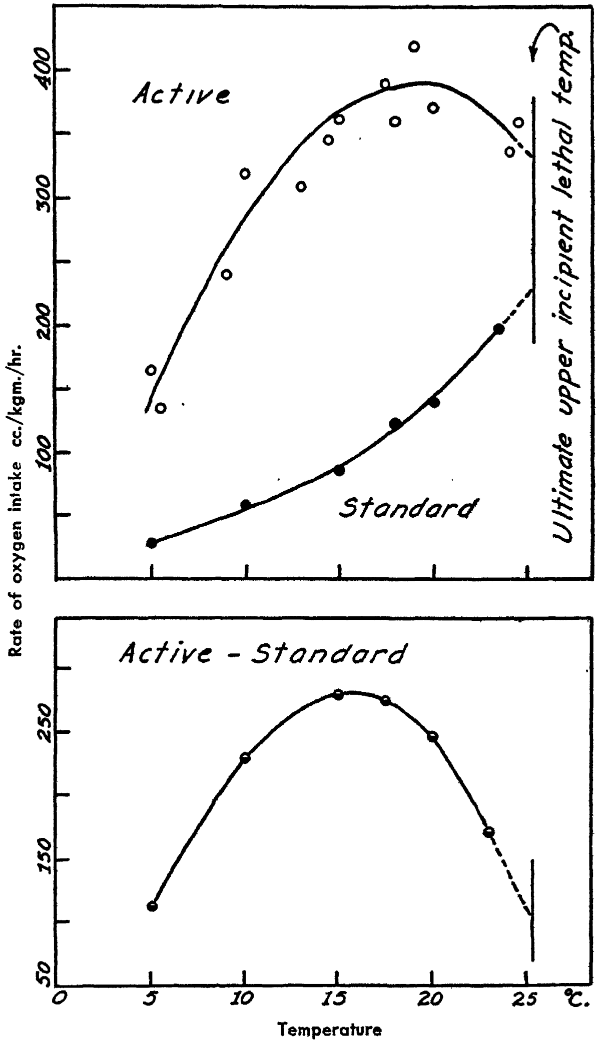

FIG. 3. *Relation of oxygen uptake to temperature in acclimated speckled trout.*
Active: Oxygen uptake of trout swimming at a maximum steady speed.
Standard: Oxygen uptake of resting trout at the lowest point in the diurnal cycle.
Active – Standard: The difference between the active and standard levels of oxygen uptake at the various acclimation temperatures.

The resting respiratory rates of three fish were measured at each acclimation temperature. At 18° C. and 20° C. it was necessary to discard data obtained from one of the three fish in each case for it was obvious from the results that these fish had remained active all night. In this connection, it will be noticed that at the peak of the diurnal cycle of the fish acclimated to 5° C. (Fig. 2) the metabolic rate of the spontaneously active fish is about the same (140 cc. per kgm. per hr.) as that of the fish stimulated to activity at 5° C. (cf. the "active" curve, Fig. 3).

The standard rates at the various acclimation temperatures are listed in Table I and plotted in Fig. 3. There is a continuing increase of standard metabolism with increasing temperature right up to the lethal level.

TABLE I

STANDARD METABOLIC RATES OF SPECKLED TROUT AT DIFFERENT LEVELS OF THERMAL ACCLIMATION

Acclimation temperature, ° C.	Weights of trout, gm.	Oxygen intake, cc./kgm./hr.	
5	17.6	39.6	Av. 27.5
	22.5	25.3	
	25.4	17.7	
10	13.3	54.0	Av. 58.7
	19.1	78.4	
	20.4	43.7	
15	16.4	91.2	Av. 85.2
	21.4	98.6	
	26.9	64.7	
18	29.7	114	Av. 123
	34.5	132	
20	17.9	152	Av. 140
	28.6	128	
23.5	20.1	199	Av. 198
	29.2	204	
	29.5	190	

Note: The standard metabolic rates at 5° C., 10° C., and 15° C. were provided by Mr. A. H. Lawrie from unpublished data.

(b) The Effect of Temperature on the Active Metabolic Rate

The maximum steady respiratory rates found for the speckled trout at oxygen pressures of 160 mm. and greater have been plotted against temperature in Fig. 3 and are listed in Table II. The rate rises rapidly with temperature, reaching a peak at about 19° C., after which it falls quite sharply. The experiments were taken to within half a degree Centigrade of the ultimate upper incipient lethal temperature found for this species, 25.3° C. (17).

(c) The Effect of Oxygen Pressure on the Rate of Oxygen Uptake at Different Temperatures

The method used for determining the active metabolic rate has the advantage that it permits a measure of respiration at decreasing oxygen

TABLE II

ACTIVE METABOLIC RATES OF THERMALLY ACCLIMATED SPECKLED TROUT IN THE INDEPENDENT RANGE OF OXYGEN PRESSURES IN RELATION TO TEMPERATURE

Acclimation temperature, ° C.	No. of trout used in one determination	Weights of trout, gm.	Oxygen intake, cc./kgm./hr.
5	2	33.3 38.2	165
5.5	3	17.6 24.6 21.4	135
9	2	35.5 31.7	241
10	3	15.6 19.0 20.1	320
13	3	17.9 19.1 15.0	350
13	2	36.8 43.2	287
14.5	1	62.3	347
17.5	2	32.8 27.6	390
18	2	74.7 27.8	361
19	2	28.7 21.3	424
20	3	22.0 23.9 18.8	371
24	3	22.5 33.3 23.8	337
24.5	3	34.4 40.3 40.7	361

concentrations. Fig. 1 is a typical plot showing the decrease of oxygen in the chamber with time. There is an initial linear phase in which the rate is independent of the level of oxygen. Following this linear phase, which is terminated in Fig. 1 at approximately 20 min., is a phase in which the rate falls in proportion to further decrease in oxygen pressure. Time can be eliminated from this diagram if tangents are drawn to the curve and the rate of oxygen uptake per unit time determined from them. These rates can be plotted against the appropriate oxygen pressure. Such rate curves are presented in Figs. 4 and 5 for the different temperatures at which the active metabolism was measured.

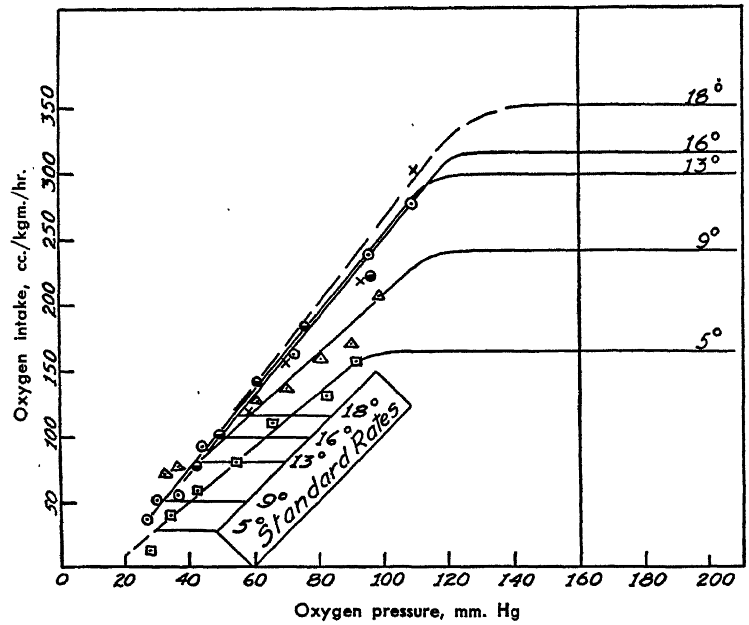

FIG. 4. *Relation of maximum steady rate and standard rate of oxygen intake to oxygen pressure at various acclimation temperatures. For the significance of the standard rates – see the text, page 285.*

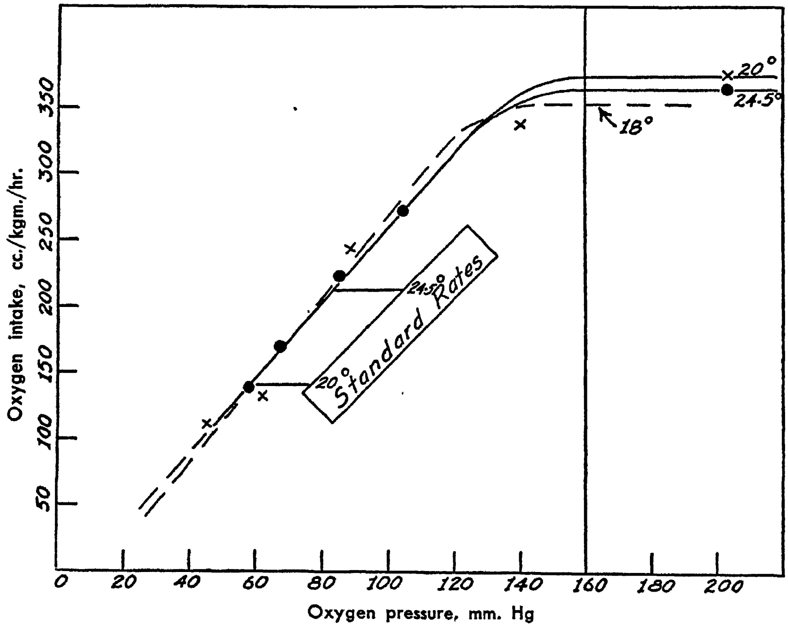

FIG. 5. *Relation of maximum steady rate and standard rate of oxygen intake to oxygen pressure at various acclimation temperatures.*

Fig. 4 shows the results of experiments at temperatures up to 18° C., Fig. 5 those at 20° C. and 24.5° C. At each temperature, the active rate of oxygen uptake remains constant down to a certain oxygen pressure. Below this pressure, the rate decreases with further decrease in the partial pressure of oxygen along a course that is approximately linear as far as the experiments were taken. In the range of oxygen pressures where the rate of respiration was dependent on the oxygen pressure, the trout at 9° C. could take up oxygen faster than could the trout at 5° C. The trout at 13° C. had a still further advantage in this respect, but at higher temperatures there did not appear to be any further increase in the rate at which oxygen could be taken up at a particular oxygen pressure in the region of respiratory dependence, as the overlapping of the curves for 13° C., 16° C., 18° C., 20° C., and 24.5° C. indicates. The pressure at which the active respiration passes from the independent to the dependent phase appears to increase with temperature throughout the biokinetic range.

(d) Lethal Oxygen Levels in Relation to Temperature

The experimental results are illustrated in Fig. 6, except for the experiment at 3° C., the survival time of each individual being represented by a point. Since the relationship in Fig. 6 between lethal oxygen level and time to death appeared hyperbolic, the tolerance levels were estimated by replotting the data, using the reciprocals of the survival times, and fitting a straight line to the points by eye. The tolerance levels were then estimated by extrapolating

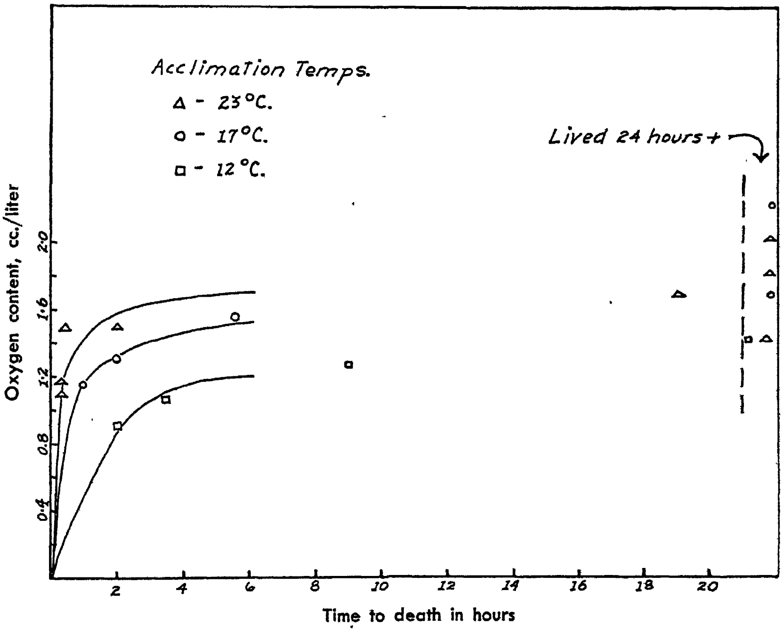

FIG. 6. *Survival times of speckled trout exposed to various low levels of oxygen.*

these lines back to $1/t = 0$. The tolerance levels thus estimated are given in Table III and are illustrated in Fig. 10. The lethal level changes considerably with temperature, being estimated at under 20 mm. Hg at 4° C. but rising to about 50 mm. at 22° C.

TABLE III

RELATION OF ACCLIMATION TEMPERATURE TO MINIMUM OXYGEN REQUIREMENTS OF SPECKLED TROUT

Temperature, ° C.	Estimated oxygen pressure allowing no excess activity, mm. Hg	Measured lethal level of oxygen pressure, mm. Hg
3.5	—	19
5	30	—
9	32	—
12	—	27
13	43	—
16	50	—
17	—	38
18	54	—
20	59	—
23	—	45
24.5	79	—

Discussion

(a) *The Effect of Temperature on the Active and Standard Metabolic Rates and their Relation to Activity*

As might be expected, the relation between standard metabolism and temperature showed no departure from the characteristics for this relationship found in other species of fish. The proportional rate of increase drops gradually with increasing temperature but the absolute rate increases throughout the range. A comparison of the proportional rates of change with those of the goldfish (16) shows no substantial difference in temperature coefficients. This observation is contrary to the suggestion of Fry (14) that the slope would be less in the case of a fish adapted for activity at low temperatures.

As is shown in Fig. 3, the course of the relationship of the active oxygen uptake differs substantially from that of the standard rate. In particular, the active rate drops with increasing temperature at temperatures above 20° C. This behavior is similar to that found for the goldfish (16) but is not universal for all fishes as the data of Paul and Fry for the bullhead (14) and unpublished data of the author for the rainbow trout and brown trout indicate. The cause for this drop in the active metabolic rate at temperatures still well below the ultimate incipient lethal is unknown. However, it is tentatively concluded that it is due to some limit being reached in the capacity of the accessory systems of the organism responsible for the marshalling of the metabolites. The course of the relation of active metabolism to temperature pehaps explains the surprising results of Montuori (25), which have never been satisfactorily explained. Montuori found in the case of three species of fish that there was no change in the rate of respiration when fish were transferred from 20° C. to 30° C. His technique was such that undoubtedly his measurements were taken when his subjects were in an excited state. Thus, it is quite possible that, in the species that he was investigating, the levels of the active rate at 20° C. and at 30° C. could have been the same, the point at the lower temperature being on the ascending limb and that at the upper temperature on the descending limb of the curves.

The active and standard metabolic rates were established primarily with a view to providing an explanation for the relation of activity of the fish to temperature such as was pointed out by Fry and Hart (16). It is presumed that the metabolism available to provide energy for external work is the difference between the active and the standard levels, neglecting the facts that the standard is not the true basal and that the active includes also the extra cost of maintaining activity. Therefore the difference between the active and standard metabolism has also been plotted in Fig. 3. This curve shows a pronounced rise and fall with a peak at approximately 16° C.

The validity of the conclusion that the scope for activity is given by the difference between the active and standard curves of oxygen uptake is supported by observations on the relation between temperature and the cruising speed of speckled trout. The cruising speed has been taken as the maximum steady speed that the fish is able to maintain indefinitely. These measurements have been made in two ways. The original apparatus used by Rogers (28) was a trough in which a current of water was generated by means of a propeller. The cruising speeds have again been measured in the same rotating chamber that was used for the determination of the active metabolism. The details of this method have been outlined by Fry and Hart (16).

Fig. 7 is a plot of temperature against cruising speed of speckled trout as measured by Rogers (28) and by the author. With respect to the curve of the latter, the data illustrated by open circles were obtained in January 1948 and those by closed circles in July 1948. Though there is some discrepancy in the temperatures at which the greatest activity occurs in the two curves drawn, they both indicate an increase of activity with temperature to between 16° and 20° C. followed by a decrease at the higher temperatures. Below the cruising speed curves the differences between standard and active oxygen intakes has been plotted at corresponding temperatures. The general similarity in shape and in the relation of slope to temperature in the two curves suggests an explanation for the change of activity with temperature illustrated. Actually, the cruising speed is more directly related to the square root of the difference between active and standard respiratory rates. This relationship has been fully discussed in connection with the goldfish by Fry (14). Such a correlation between activity and oxygen intake has been similarly worked out by Hill *et al.* (18) for man and by Chadwick and Gilmour (5) for *Drosophila.*

Hill subtracted the resting oxygen intake of the subject from the oxygen consumption as he walked and ran at various speeds and obtained a straight line relationship between this difference and a power of the speed. Chadwick and Gilmour obtained an increase of oxygen intake over the resting rate that varied directly with the square of the wing rate of *Drosophila.*

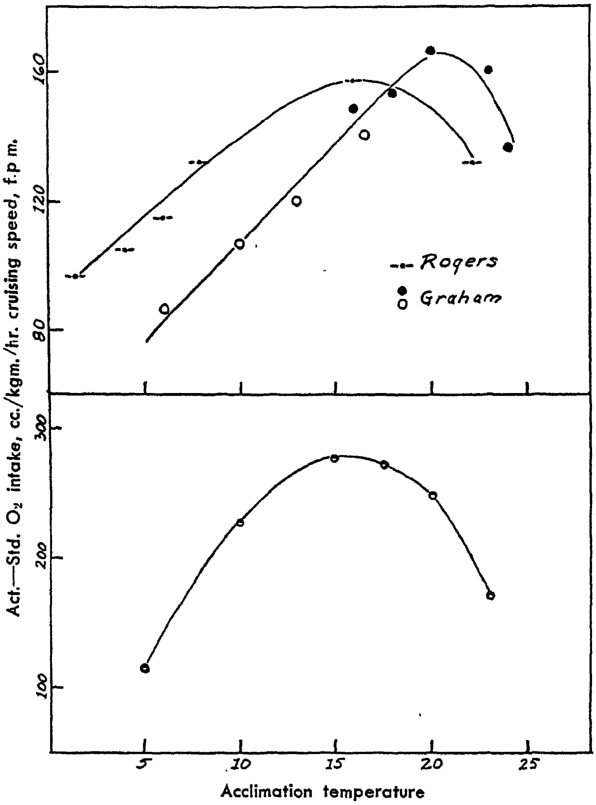

FIG. 7. *A comparison of the maximum speed at which speckled trout can swim steadily at various temperatures, and the difference between the active and standard levels of oxygen intake.*

(b) The Effect of Oxygen Pressure on the Rate of Oxygen Uptake at Different Temperatures and Its Relation to Activity

The phenomenon displayed in Figs. 4 and 5 is, of course, well known and has been, in general, referred to as respiratory dependence. Similar data have been recently presented for the salmon, the roach, and the pike by Lindroth (24) and more completely for the goldfish by Fry and Hart (16). There are numerous earlier but fragmentary examples.

The interpretation suggested for the nature and order of the curves is as follows. During the dependent phase, some rate in the process of uptake and transport of oxygen to the site of respiration is being limited by the oxygen supply. At the temperatures of 5° C. to 9° C. and 13° C. the rate of supply at any given pressure in the dependent range is presumably improved by an increase in pumping rate of the ventilatory apparatus. At higher temperatures, apparently, the trout has not this advantage perhaps because of the high cost of respiration when water is the respiratory medium.

It is noteworthy that the oxygen uptake – oxygen pressure curves do not pass through the origin. The uptake approaches zero at an oxygen pressure of about 20 mm. Hg. Lindroth (23) noted a similar circumstance. He suggested that the pressure of oxygen at which the uptake fell virtually to zero corresponded to the unloading tension of arterial blood. This does not appear to be the whole explanation in the case of the speckled trout since 20 mm. Hg of oxygen should be sufficient to saturate the blood of speckled trout at the highest temperature investigated (20). Undoubtedly therefore the dynamics of the situation also enter into the case. The original oxygen content of water presented to the respiratory surface (20 mm. Hg) must be soon taken up by the blood in the lamellae and the pressure so quickly reduced that equilibrium is established at a pressure much lower than 20 mm.

At levels of oxygen where the active metabolic rate is reduced there must be some limiting of overall activity. An experiment to illustrate this effect in the case of the speckled trout is shown in Fig. 8. In this experiment the oxygen pressure of the water in the rotating chamber was adjusted to various levels by bubbling various nitrogen – oxygen mixtures through it. The cruising speeds of three individual speckled trout were measured at each of these various levels of oxygen pressure with the results indicated in the figure. At oxygen pressures below 80 mm. Hg there is a steep falling off of the cruising speed. The agreement between this experiment and the relation between the

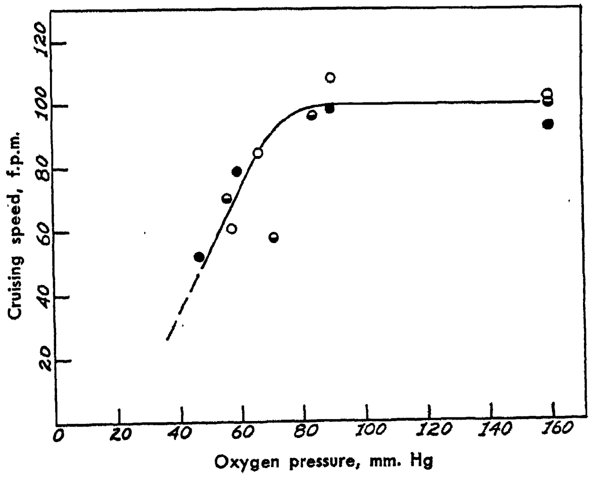

FIG. 8. *The maximum steady speeds that a speckled trout acclimated to 8° C. can maintain at that temperature in water containing various partial pressures of oxygen.*

rate of oxygen uptake and oxygen pressure is only approximate. At the temperature in question (8° C.) the respiration would have been expected to become dependent upon oxygen pressure at 100 mm. Hg. However, the relatively short duration of the experiments makes it probable that the cruising speeds obtained at the lower pressures were in excess of those that would represent a true steady state.

The general order of reduction of activity consequent on reduction of oxygen pressure below air saturation is illustrated in Fig. 9. This figure depicts the degree to which the active metabolism in a steady state could rise above the standard requirements. The profiles given have been expressed in per cent air saturation rather than mm. Hg pressures since the former terminology has been almost universal in fisheries biology.

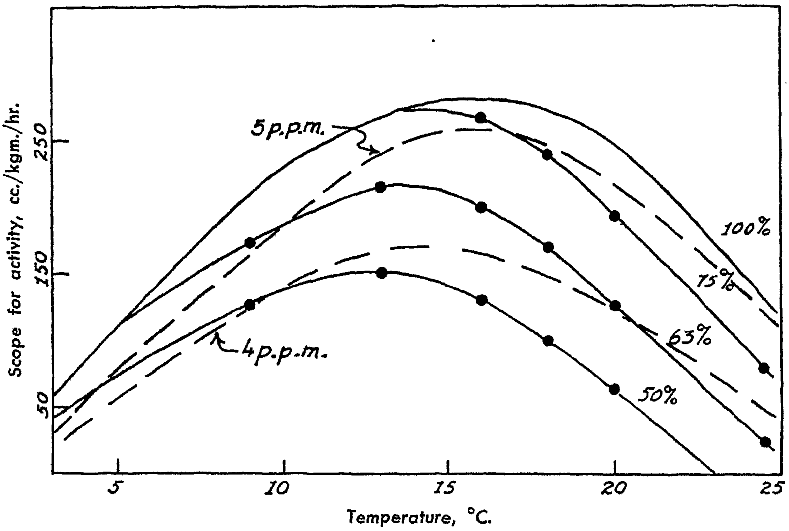

FIG. 9. *Relation between temperature and the scope for activity of speckled trout in waters of different percentages of air saturation. The two broken lines indicate the scope at 4 and 5 p.p.m., the range of oxygen that field workers have indicated as being the safe minimum for speckled trout.*

From the point of view of providing sufficient oxygen to allow the speckled trout its full scope for activity it would seem from Fig. 9 that less than 75% air saturation would be detrimental to speckled trout at any temperature. Above 20° C. even reduction to 75% would be serious. This is in strong contrast to the requirements of such a fish as the goldfish in which reductions down to 25% air saturation have no effect. Two other levels of saturation, 63% and 50%, are shown in Fig. 9. The 63% line indicates that even at the ultimate lethal temperature death might not be due to oxygen lack. This value is in agreement with Brett (3) who found by experiment that an oxygen content down to 62% air saturation in the waters of his lethal baths did not affect the rate of dying. The 50% saturation level affects the scope for activity over the whole biokinetic range of the speckled trout and would bring death by anoxia at a temperature somewhere between 20° C. and 25° C.

These laboratory findings are thus in close accord with the empirical level of 4 to 5 p.p.m. taken from field observations at summer temperatures. Five parts per million represent respectively 62, 70, 80, and 85% air saturation at temperatures of 10°, 15°, 20°, and 25° C. Thus this level represents almost exactly the safe minimum suggested by the experimental data. Four parts per million (50% air sat. at 10° C., 60% sat. at 20° C.) would appear to be definitely below the minimum for full activity.

(c) Lethal Oxygen Levels in Relation to Temperature

It has been proposed by Lindroth (23) and Fry (14) that the oxygen level at which the rate of oxygen uptake is reduced to no more than the requirements for standard metabolism can be taken as the minimum requirement for the existence of the organism. The standard levels of metabolism have been indicated on Figs. 4 and 5 by short horizontal lines. According to the above reasoning, the minimum oxygen levels for existence of speckled trout would be described by the oxygen pressure at which these horizontal lines cut the appropriate oxygen uptake – oxygen pressure curves. Thus in Fig. 4 at 5° C. the level of standard metabolism is that which occurs on the active oxygen uptake curve at 30 mm. Hg. This point was termed by Fry (14) as the "level of no excess activity". The levels of no excess activity are given in Table III for the various temperatures investigated and are illustrated in Fig. 10.

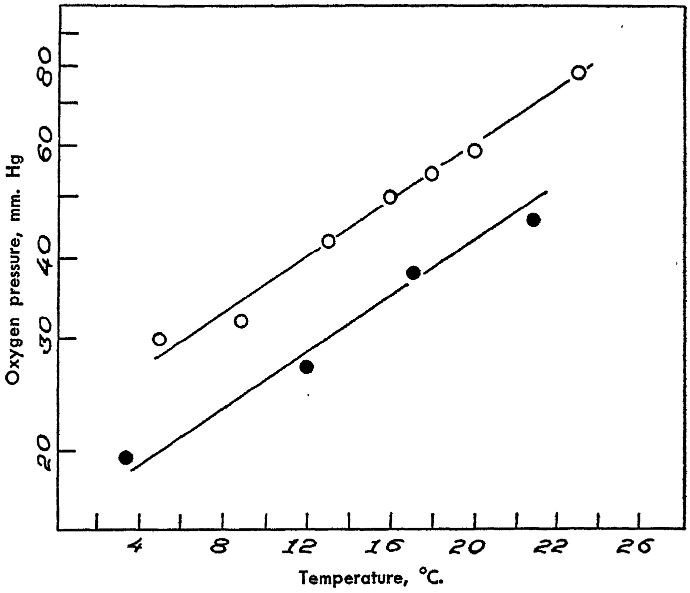

FIG. 10. *The relation of the level of no excess activity (open circles) and the lethal level of oxygen (solid dots) to temperature.*

The level of no excess activity changes considerably with temperature. At 4° C. it is approximately 26 mm. Hg while at 22° C. it is about 70 mm. Hg. Thus at 22° C., if the index is appropriate, the speckled trout requires water that is approximately 40% air saturated in order to exist without undertaking any appreciable amount of external work.

The experiments to measure the lower lethal levels of oxygen were carried out in order to test the validity of the estimates of the levels of no excess activity as measures of the lower levels of tolerance for oxygen. The level of no excess activity might give an erroneous value for one of two reasons. The estimate might be far too high because the standard rate of metabolism measured might include far too much expenditure for the maintenance of chance movements, or it might be unduly low because the standard rate was measured under conditions of high oxygen and the cost of ventilation would thus be at a minimum. When the oxygen level was reduced to the neighborhood of the level of no excess activity much more water would have to be pumped over the gills to supply the oxygen required to maintain the standard metabolic rate and the work thus involved might considerably increase the basal requirements of the animal.

The direct estimates of the lethal oxygen levels are compared with the levels of no excess activity in Fig. 10. Although the data are meager the lethal levels bear a consistent relation to the levels of no excess activity throughout the whole of the biokinetic range. The fact that the lethal levels are lower than the levels of no excess activity would seem to indicate that the activity was not completely eliminated when the measurements of standard metabolism were made, the first of the two sources of error mentioned in the paragraph above. The level of no excess activity is therefore a conservative estimate of the lower limit to which the oxygen content can be reduced before it becomes too low for these speckled trout.

Under summer conditions when the fish are active, any drop in the oxygen content that is enough to reduce the maximum rate of oxygen uptake to any appreciable degree must be considered detrimental to the organism. However in winter when the call for activity is not so great, it may be possible for the speckled trout to live under conditions of oxygen that approach much more nearly to the lethal level. It is interesting therefore to note that the speckled trout can probably live in waters that have a considerable oxygen deficit in winter, at least where buffering is sufficient to prevent an undue rise in the free carbon dioxide. At 4° C. the level of no excess activity is 16% of air saturation (1.5 cc. per l.). The estimated lethal level at the same temperature is about 1 cc. per l. or 11% of air saturation. These estimates assume of course that no other harmful products of decomposition are present, but on the other hand they also make no allowance for acclimation to the gradually lowering oxygen content that would probably occur in nature.

Acknowledgments

This work was carried out in the Ontario Fisheries Research Laboratory of the Department of Zoology, University of Toronto, under the direction of Prof. F. E. J. Fry. It was supported by grants to Professor Fry from the National Research Council of Canada and the Research Council of Ontario. The experiments on standard metabolism were carried out by Messrs. A. H. Lawrie and E. S. Gibson, and the permission to use their unpublished data is gratefully acknowledged. The writer is also indebted to Miss I. Chuhaldin and Mr. J. MacDougall for assistance in experiments. The fish used in these experiments were provided by the Department of Lands and Forests of Ontario.

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STUDIES OF WATERFOWL IN BRITISH COLUMBIA

BALDPATE[1]

By J. A. Munro[2]

Abstract

A relatively large population of *Mareca americana* winters on the Coastal Plain, almost exclusively along the coast line when meadow flood ponds and sloughs farther inland are sealed by frost. A winter population in the interior is small in numbers and irregular in occurrence. Nesting grounds are in the interior only, from the Okanagan Valley and Kamloops–Nicola region, where the species is scarce, to as far north at least as the Haines Road in the northwest section of the Province. The most productive nesting grounds are in the Cariboo Parklands and the Lakes District near Vanderhoof. The main spring migration of transients through the central interior takes place during the last two weeks of April and by early May a breeding population is established on the many sloughs and ponds of the nesting range. Dry *Carex* meadows bordering certain water areas is the preferred nesting habitat. It is usual for the full clutch of eggs to be laid not later than June 15. In the Cariboo Parklands the average number of young in 10 June broods was 7.0, the average of 75 July broods was 6.2, and the average of 14 August broods was 5.2. In the Lakes District near Vanderhoof the average number of young in 37 broods during July and August was 7.0. Females with broods habitually frequent the open water of marsh ponds, lake bays, and marsh-edged rivers. Here, if approached by man, they vigorously defend their young by ruses to attract attention from them. Short, successive flights in front of a moving canoe, and splashing across the water in a direction leading away from the young are examples of behavior at this time. Postbreeding males associated in flocks on open water often accompany rafts of postbreeding coots and diving ducks. Aquatic insects is the chief item in the diet of downy young. Adults feed principally on the foliage of aquatic plants, grasses, algae, and seeds of aquatic plants, in that order. Insects are eaten in relatively small amounts. The baldpate may, under certain conditions and in certain localities, destroy cultivated forage plants. It is an important game duck and, because of its almost exclusive vegetable diet, highly palatable. Revenue derived from the hunting of baldpate, and the economic value of its flesh for human food, more than compensate for any economic loss resulting from crop destruction.

Introduction

In British Columbia the baldpate, *Mareca americana* (Gmelin), probably occupies fourth place in numerical status among the pond duck group of the Anseriformes. Winter populations occupy suitable habitat along the Pacific Coast from southeastern Alaska to Mexico, and in British Columbia a summer population is widely distributed through the central interior. It is not known to nest west of the Cascade and Coast mountain ranges. Recent information concerning the baldpate's breeding status in the Pacific States is lacking but apparently small numbers nest east of the Cascade mountain range as far south as Malheur Lake in southeastern Oregon and in Modoc County in northeastern California (3, 4).

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Contribution from Dominion Wildlife Service, Department of Mines and Resources, Ottawa, Canada.

[2] *Dominion Wildlife Officer for British Columbia.*

The facts of distribution, migration, and life history assembled in this paper represent the sum of field observations from 1911 to 1948, to which is added the small amount of data obtained from the banding of 306 baldpate, chiefly at Station 1 (See Table I). For explanation of the banding terms used see an earlier paper in this series (6).

TABLE I

NUMBER OF BALDPATE BANDED IN BRITISH COLUMBIA AND TOTAL RECOVERIES

Banding periods	Number banded			Totals	Totals recoveries	Recovery, %
	♂	♀	Not sexed			
Lulu Island						
1924, Mar. 15–Apr. 7	0	0	3	3	1	33.3
Station 1						
1928, Oct. 20–Dec. 1	1	0	0	1	0	
1930, Oct. 21–Dec. 5	0	1	0	1	0	
1931, Nov. 3–Dec. 9	3	6	0	9	0	
1932, Oct. 16–Dec. 9	11	16	0	27	5	18.5
1933, Mar. 29–Apr. 18	3	1	0	4	0	
1933, Oct. 4–Dec. 12	40	49	0	89	13	14.6
1934, Jan. 28–Mar. 14	6	7	0	13	2	15.3
1934, Oct. 7–Dec. 5	13	30	15	58	15	25.8
1935, Mar. 5–Apr. 11	0	0	0	0	0	
1935, Oct. 22–Dec. 31	0	6	0	6	0	
1936, Jan. 18–Apr. 11	0	1	0	1	0	
1936, Oct. 10–Dec. 28	2	4	0	6	1	16.6
1937, Mar. 19–Apr. 4	1	1	0	2	0	
1937, Oct. 31–Dec. 12	3	5	0	8	2	25.0
1938, Nov. –Dec. 31	0	0	22	22	2	9.1
1939, Jan. 1–Apr. 19	0	0	0	0	0	
1939, Nov. 14–Dec. 31	4	16	0	20	3	15.0
1940, Jan. 1–Mar. 31	10	10	0	20	0	
Station 2						
1933, Mar. 14–Mar. 29	3	1	0	4	0	
1934, Feb. 18	0	1	0	1	1	100.0
1935, " 11	0	1	0	1	0	
Station 5						
1933, Sept. 7–Oct. 6	0	0	10	10	1	10.0
Totals	100	156	50	306	46	

Total recoveries 15.03%

Distribution and Seasonal Movements

COAST REGION

Autumn and Winter

Little precise information concerning autumn migration dates of the baldpate is available. The arrival of the main flights vary in time to a greater extent than do the flights of other pond ducks. In some years large numbers have been recorded in November, as in 1938 when 500 were counted at Sooke Harbour, Vancouver Island, on Nov. 14.

In winter the British Columbia coast population is distributed from the Queen Charlotte Islands to Sooke Harbour and Esquimalt Harbour on the south end of Vancouver Island. Some of the many islands in Georgia Strait, the many inlets on the mainland coast, the Fraser Delta, the Pitt River region, and Boundary Bay are also populated to a greater or less degree. Part of the population appears to inhabit the sea littoral almost exclusively, another part uses sheltered bays of the sea chiefly as resting places and travels daily to feeding grounds on the fields, some of which are considerable distances inland. Other elements of the population are more or less sedentary on inland areas of the Coastal Plain but the size of these populations and their movements are modified in accordance with the amount of flood water on the fields. In dry years the numbers are small; in years of heavy precipitation the numbers may be large, but, as with other pond ducks, a sudden drop in temperature and consequent freezing of sloughs and flood ponds causes an immediate exodus of the population to the coast.

Selected winter counts are: Victoria Region, Jan. 5, 1940 - 266, Jan. 5, 1942 - 400±, Feb. 23, 1946 - 600±; Esquimalt Lagoon, Feb. 28, 1934 - 1000±; Comox, Dec. 20, 1925 - 300±; Westham Island, Dec. 4, 1934 - 800±.

More recent estimates of local winter populations, obtained during the January waterfowl inventory, follow:

	1947 Jan. 7 to 17	1948 Jan. 7 to 10	1949 Jan. 11 to 14
Fraser River mouth to Boundary Bay	7590	1060	3151
Southern Vancouver Island	4300	2055	4441
West Coast Vancouver Island	—	1800	—
Graham Island	300	100	400

From October, 1928, to Mar. 31, 1940, a total of 284 baldpate was banded at Station 1. Recoveries numbered 43, of which 33 represent birds shot, and 10 represent birds retrapped and released. Thirty-two are current recoveries from the Coastal Plain taken one day to three months after the date of banding and are distributed as follows: 17 at, or within a 12 mi. radius of, Station 1; one at Boundary Bay; eight at intermediate points; and six in Washington State from Sumas to Bellingham. Details of the remaining 11 recoveries of birds banded at Station 1, and three recoveries from birds banded elsewhere, are shown in Table II.

While it has seemed desirable to put these banding data on record the number of returns is not sufficient for detailed analysis. The high percentage of local current recoveries and the late dates on which they were recovered suggest that most of them represent baldpate that were wintering on the

TABLE II

RECOVERIES OF BALDPATE BANDED IN BRITISH COLUMBIA

Banding station	Date banded	Date recovered	Locality where recovered
Station 1	Nov. 20, 1932	Jan. 13, 1935	Jones Is., Haro Strait, B.C.
	Nov. 20, 1932	Jan. 30, 1934	Station 1
	Nov. 5, 1933	June 15, 1936	Fort Yukon, Alaska
	Nov. 5, 1933	Nov. 22, 1934	Skagit Co., Wash.
	Nov. 13, 1933	Nov. 30, 1934	Whatcom Co., Wash.
	Nov. 13, 1933	Jan. 1935	Sumas Prairie, B.C.
	Dec. 7, 1933	Nov. 2, 1934	Skagit Co., Wash.
	Jan. 30, 1934	Nov. 2, 1934	Skagit Co., Wash.
	Nov. 28, 1934	Dec. 3, 1935	Comox, V.I., B.C.
	Nov. 2, 1937	Dec. 4, 1938	Dewdney, B.C.
	Dec. 29, 1939	Dec. 10, 1940	Sardis, B.C.
Lulu Island	Apr. 2, 1934	Jan. 9, 1935	Lulu Island, B.C.
Station 2	Feb. 18, 1934	Oct. 4, 1935	Wembley, Alta.
Station 5	Oct. 6, 1933	Nov. 15, 1933	Skagit Co., Wash.

Coastal Plain. It will be noted that all autumn and winter recoveries in later years also are from the Coastal Plain, among them are one recaptured at Station 1, one shot at Sardis near Station 1, and one shot near Chilliwack about six miles distant.

Spring Migration

During late February and in March the winter population is increased by the arrival of transients from more southern latitudes. At this time disturbance to the normal activities of pond ducks caused by hunters has ceased so that many flooded fields adjacent to highways, which earlier in the season were avoided during daylight, now attract many pond ducks including baldpate (Fig. 1). Sample spring counts are: Somenos Lake, V.I., Mar. 3, 1939 – 300 ±; Quamichan Lake, V.I., Mar. 10, 1934 – 75; Victoria, V.I., Apr. 24, 1940 – 84; Boundary Bay, Mar. 23, 1922 – 750 ±; Mar. 15, 1934 – 200 ±. By the end of April the last of the spring flocks usually have migrated.

Interior Region

Winter

Baldpate winter irregularly, and usually in small numbers, in the Okanagan Valley and on the North Thompson River but not elsewhere in the interior so far as known. The small lakes and ponds that constitute their feeding grounds in spring and autumn are frozen even in mild winters; so also as a rule are the shallow margins of the large, deep lakes. Deprived through this circumstance of access to food by dipping, baldpate commonly frequent open water in the company of diving ducks and American coots, *Fulica americana*, and are largely dependent upon them for food. In another section of this paper the baldpate – diving duck relationship is discussed in some detail.

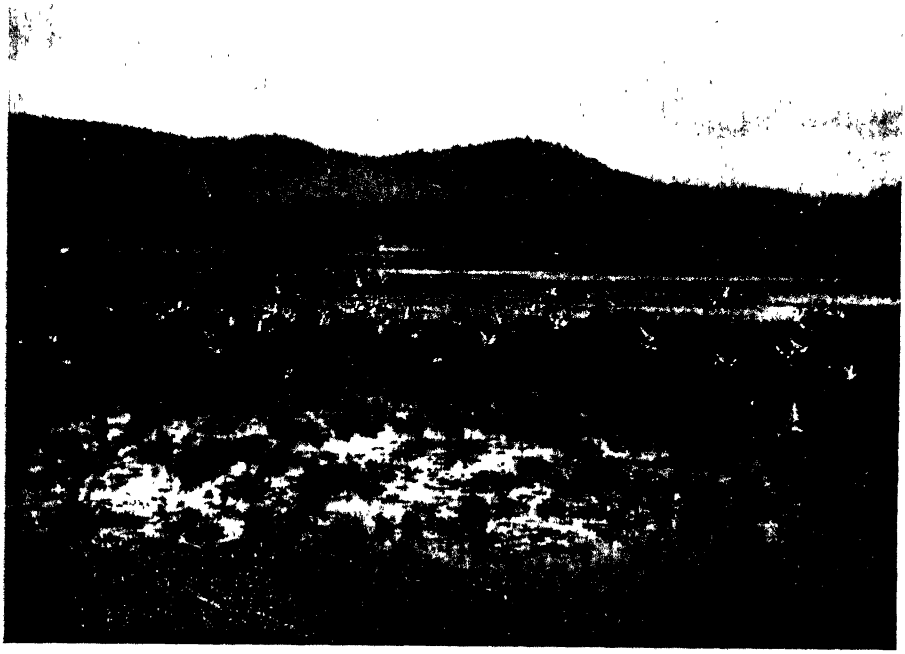

FIG. 1. *Baldpate on Quamichan Lake – Vancouver Island.*

Through the period 1911-1924 the population of baldpate wintering at the north end of Okanagan Lake was quite small, the largest number counted at any time being five observed frequently through January, 1918. More recently larger numbers have been recorded. Thus in the winter of 1938-39 when redhead, *Aythya americana*, and a smaller number of greater scaup duck, *Aythya marila*, wintered, a relatively large number of baldpate accompanied them through December and January at least. Precise counts are Dec. 28 – 160, Jan. 28 – 120. Observations were not continued beyond the last date so it was not learned what number, if any, remained through the balance of the winter. On Dec. 21, 1944, Swan Lake was frozen except for some 20 ac. on the east side; this open water attracted numerous diving ducks, and accompanying them on that date were 325 ± baldpate.

More recent observations made on Jan. 18, 1949, are of small numbers on the North Thompson River near Chase, viz.; four baldpate with 30 greater scaup duck, five with 250 ± greater scaup duck, and 26 in company with 20 mallard, *Anas platyrhynchos* (D. A. Munro in litt.).

Spring Migration

The spring migration of baldpate through the Okanagan, Nicola, and Kamloops regions takes place in late April, usually following the main flights of pintail, *Anas acuta*, and green-winged teal, *Anas carolinensis*. It is usual for them to travel in small flocks sometimes numbering 10 or less; the following are counts of some of the largest units tallied during the spring migration in recent years. Okanagan Lake, Mar. 28, 1939 – 39; Pond near Vernon, Apr. 18, 1939 – 40, Apr. 18, 1940 – 20, Apr. 18, 1941 – 21, Apr. 25, 1947 – 26; Rawlings Lake, Apr. 12, 1940 – 30. On certain lakes, used as feeding and resting places during migration, it is customary for a number of units to band together while they remain on these waters, as on a relatively large lake on the Vernon Commonage where 138 were counted in one flock on Apr. 16, 1948. Munson's Lake, near Kelowna, is another favored resting place where 400 were tallied on Apr. 23, 1938.

Observations of the spring migration in the Cariboo Parklands were made in April of 1940, 1941, and 1943. In the first year baldpate were not plentiful up to and including Apr. 24 when the study for the spring season was concluded. On that date the total tallied on two lakes was 37 and it was assumed that the main migration was still to come.

In 1941 during Apr. 15 to 17, the first three days of the study, only two flocks, each made up of 30 birds, were tallied. All of one flock, immobile on deep water 300 yd. from the shore of Lac la Hache, were obviously newly-arrived transients resting after a long journey. On Apr. 18 the transient population on the key lakes of the region had increased substantially, small flocks being sighted on most of the ponds and lakes visited. On Apr. 22 approximately 200, in units up to 40, alighted on 105 Mile Lake; on 149 Mile Lake a total of 80, and on Cummings Lake a total of 36, were counted.

In 1943 flocks containing a maximum of 40 individuals were counted in the Nicola Region on Apr. 27, at Clinton on Apr. 28, and at Lac la Hache on Apr. 30. The last had increased to 82 by 4.00 p.m. on that date. At Williams Lake at 11.30 a.m. on May 3, 20 birds in one flock were asleep on the water. The last, a flock of 20, to be identified as of the transient population, alighted on a pond near 103 Mile Lake on May 5.

Little information concerning the spring migration through the eastern interior is available. On the Kootenay Flats in early May, 1948, the few baldpate seen, viz.; May 10 – 4, May 15 – 8, May 17 – 4, were identified as late migrants; none are known to nest in the region.

Autumn Migration

An early southern flight of baldpate in the interior has frequently been observed. Thus, a flock of 200 ± was counted on a slough in the Lakes District near Vanderhoof on Aug. 22, 1945, and by the end of the month this flock numbered 400, approximately. Flocks totalling 150 were counted on a series of sloughs in the Okanagan Valley on Sept. 12, 1939, and 100 were counted at the same place on Sept. 12, 1940. The main migration takes place some time during October and November, with notable variation in time and in numbers. This irregularity is illustrated by the following estimates of maximum numbers tallied in each of 11 years at Swan Lake.

1933, Nov. 4 – 150
1934, Oct. 4 – 500
1937, " 1 – 400
1939, " 28 – 2500
1941, " 15 – 90
1942, Nov. 5 – 300

1943, Oct. 6 – 600
1944, " 30 – 200
1945, " 10 – 30
1946, Nov. 13 – 225
1948, " 13 – 1200

On the Kootenay Flats in 1947 the first transients, a group of four, appeared on Aug. 12, and later arrivals were recorded as follows: Aug. 25 – 7, Aug. 26 – 500 ±, Sept. 10 – 50, Sept. 11 – 200 ±. In 1948 the tally of incoming transients was: Aug. 11 – 20 ±, Aug. 27 – 235, Aug. 28 – 275 ±, Sept. 16 – 120.

Reproduction

Two broods of baldpate counted at Swan Lake, Okanagan, July 31, 1940, and one brood observed at Hamilton Corrals in the Nicola District, Aug. 10, 1939, represent the most southerly breeding records in the Province. A brood of young seen at Mile 85 on the Haines Road is the most northerly one (T. N. Shortt, MS.). Relatively large populations inhabit the Cariboo Parklands (8), the Chilcotin Region (11), the Lakes District near Vanderhoof, where in 1945 it was the commonest nesting duck (10), and the Peace River Parklands, where in 1938 it outnumbered all nesting ducks except the mallard (2). Smaller populations have been studied on several lakes near Francois Lake and in lakes along the Bulkley River as far west as Smithers (9).

Baldpate arrive in the Cariboo Parklands during the latter part of April, some already being mated at the time of their first appearance on the lakes and sloughs of that region. For example, on Apr. 22, 1941, each of 8 or 10 mated pairs among several flocks of restless baldpate were conspicuous by reason of their relative immobility and constant close companionship. By the first week in May all but a few of the transients have left; the nesting population is assembled in small flocks, and there is much excited activity among them. It is common to see one or more males on the wing, in pursuit of a female, circle the pond that is their temporary home, and, after a short flight, drop to the water again. Nuptial flights of mated pairs around pond or slough also take place at this time. In the air the female utters a soft call-note, repeated numerous times in quick succession—a note identical in quality and volume to the alarm note given when a female is concerned about the safety of her young. The whistle of the male is also heard during the nuptial flights. Where a number of pairs are in joint possession of a pond they maintain fairly close association with one another and assemble in a flock when danger threatens. None seem to frequent or defend any particular feeding and loafing territory. By the first week in June the nesting population is widely distributed and the majority of females are either laying or incubating.

In the Lakes District, Vanderhoof, during the mating season of 1945 males outnumbered females and it was common to see groups of three or four males and one or two females circling the marshes. Sometimes females were accompanied by three or four males and mated pairs in courtship flight were joined by a second male.

Nesting

Baldpate seem to show preference—perhaps a group tradition—for nesting beside certain lakes while avoiding others in the same locality that have almost identical cover and food. For example, the species nests, sometimes commonly, at 103 Mile Lake but none do so at the adjacent and similar 105 Mile Lake (8). The preferred habitat is one of marshy sloughs in the grasslands, the site usually is in the dry *Carex*, that, in such places, forms an outer belt of vegetation around the marsh proper.

A nest with eight eggs found at Sorensen Lake, June 4, 1941, was situated in a 30-ft. strip of cultivated brome grass between a ploughed field and a marshy lake shore, the site being a tall brome grass clump 10 ft. from the edge of the ploughing and 100 ft. from the edge of the marsh. The nest was constructed substantially of straw and a small amount of down, which was added to during the week following. On the several occasions on which the nest was visited, the female flushed at about 10 ft., fluttered low over the top of the grass for a few yards, then flew to the lake.

A nest at Cummings Lake built beside a flat rock on a dry, open slope, 20 ft. from the lake shore, which at this point was fringed with round-stem bulrush, measured 8 in. in diameter, and was partly concealed by a tall clump of fine grass. A high rim of down surrounded the seven eggs that were within a day or so of hatching on July 17, 1948. When flushed from the nest the female alighted on the lake at a point about 40 yd. distant.

Two nests were examined in the Lakes District, Vanderhoof. The first, made of desiccated sedge stems and situated among sedges 60 yd. from the edge of a marsh, contained eight eggs that were destroyed by crows prior to June 17 (1945). The second, concealed in thick vegetation near the margin of a brush-edged stream, contained eight eggs on June 13 (1946). This nesting was successful.

Brood Survival

Investigations of baldpate nesting populations in the Cariboo Parklands were carried on in each of the years from 1936 to 1943 with the exception of 1940. During this period a total of 99 females accompanied by broods was tabulated. The earliest date for the appearance of downy young was June 18, 1941; the average number of young in 10 broods counted in June was 7.0, the average number in 75 July broods was 6.2, and the average number in 14 August broods was 5.2.

In the Lakes District, near Vanderhoof, in 1945, the earliest date for the appearance of downy young was July 4; the average number of young in 37 broods counted through July and August was 7.0; in 1946 the average number in eight broods was 6.6; the earliest date for downy young was July 3.

In the summer of 1948 a Provincial Game Commission party working in the Chilcotin Region reported a total of 26 broods counted between July 5 and July 21 in which the average number was 6.7 (11).

At Francois Lake in August, 1944, the average number of young in four broods was 5.3; a larger number of young was associated with females in a single band that totalled 70. In July, 1946, the average number in five broods was 6.6.

In the Bulkley Valley, at La Croix Lake and Covert Lake, July, 1944, the average number in five broods was 7.0, and in a backwater of the Bulkley River near Smithers, July 17, 1944, a female accompanied a brood of 10.

Behavior of Females with Young

Female baldpate with broods of young are given to frequenting open water and it is common to see them swimming along the marshy edges of lakes and rivers. As in studying certain waterfowl it often is desirable to paddle a canoe along the margin of whatever waters are being investigated, this procedure afforded many opportunities to observe the fear reaction of the female baldpate in the presence of man. Their behavior under these conditions follows the same general pattern. Usually the duck will rise when the canoe is sighted, fly for a short distance over the water, then turn inshore and alight close to the canoe. Should the canoe be kept in motion the duck quickly takes wing again and, in a succession of short flights with intervening periods on the water, attends the progress of the canoe. A soft alarm note is uttered frequently. Meanwhile the young, if they happened to be outside the marsh cover when the female left them, scamper over the water or swim less hurriedly to the shelter of the adjacent marsh. This behavior of the female has been observed when a brood was visible and when a brood was hiding in thick cover. In some instances when the brood was in plain sight on the water the female rushed across the surface for a short distance, with neck extended horizontally and wings threshing the water, before beginning the succession of short flights in the manner described. Occasionally also one will rejoin her brood after a demonstration of this sort that has continued but for a few minutes. More often, however, she will remain in the vicinity of the canoe. On one occasion, Williams Lake, Aug. 2, 1937, one continued the successive flights for two hours while a canoe was being paddled around the two mile circumference of a marshy bay. At the same place, on July 26, 1943, a female that had her brood hidden in the shore-lined marsh flew out and circled the canoe, then alighted on the water close by. As I paddled on she followed, or preceded, the canoe in a series of short flights and called continually. About one quarter-mile farther on a second female flew out of the marsh to join the first and behaved in the same manner. The two remained together and followed the familiar pattern of behavior for the next half-hour. Many similar instances could be cited.

Occasional departures from this common behavior have been observed. Thus, at 103 Mile Lake, July 18, 1939, where two females were leading their combined broods, one demonstrated in the usual manner while the other led the 18 young into the nearby bulrush marsh. Another female, which when first seen was swimming several hundred yards away from her brood of eight, took wing, circled the canoe several times, then dropped to the water. A moment later she flew directly to the young and led them into the marsh. In the Lakes District near Vanderhoof the banding together of several females and their broods was noted frequently, for example, on July 24, 1945, when one band of five females and 38 young, and another two females and 16 young were counted.

On Cummings Lake, June 30, 1942, a brood of eight young about one-fifth grown rushed out from the marshy shore to open water; the female, swimming about 30 yd. away, rose at once and, dropping again to the water immediately behind the young, urged them forward by rushing back and forth across the surface, sometimes standing upright and splashing with her wings. At 103 Mile Lake, July 12, 1942, a female with three large young acted in an identical manner. At the same lake a little later a small downy baldpate swam out from the rushes, joined a brood of three buffle-heads and with them swam off behind the foster mother. On Nulki Lake, near Vanderhoof, a quite different behavior was general; it was usual for the female to swim ahead of the brood and, if pressed, lead them in a rush across the water toward cover on shore (10).

Females accompany their broods until the young are nearly full-grown, sometimes after they have reached the flying stage. No information concerning the flightless period of the female has been obtained other than the obvious fact that the flying stage occurs some weeks later than it does with males.

Postbreeding Behavior of Males

Most of the females are incubating by the third week in June and the males have left them. The latest dates on which mated pairs were observed are June 22, 1941, and June 21, 1945. Some males associate in small bands that may include a few females, presumably those that for one reason or another have failed to mate or that have nested unsuccessfully. As both males and females are sexually mature in their second year the number of nonbreeding birds in any population is not large. Other males, in this early midsummer season, associate with postbreeding mallard and pintail and may continue to do so for several weeks. Still others band together in much larger flocks that may join rafts of postbreeding diving ducks on open water. Early records for flocks of postbreeding males are: Elliot Lake, June 12, 1942 – 18; 103 Mile Lake, June 16, 1942 – 5. By the first week in August the majority, in full or partial eclipse, are flightless – a condition that lasts about three weeks.

The number of postbreeding males present on any particular series of sloughs does not necessarily indicate the size of the local nesting population as there is considerable movement from one district to another. Thus at Minnie Lake, situated in the Nicola Region, where a few baldpate nest, a total of 60 males was counted on Aug. 10, 1938. Other instances of a southern movement of postbreeding males have been noted. How far south these movements extend is not known.

The fluctuation in numbers from year to year, so apparent in transient flocks and winter populations composed of both sexes, is noted also in the postbreeding flocks of males on the nesting grounds. For example, in the Cariboo Parklands considerably more postbreeding males were tallied in 1937 and 1939 than during the three succeeding summers.

The following table of enumeration shows the periods in which postbreeding males are most conspicuous, and the variation in size of flocks.

Flightless males in eclipse seem less inclined to seek concealment than do the males of other pond ducks when in this condition and are frequently prominent in rafts of postbreeding diving ducks. When alarmed they flap across the water sometimes to a distant part of the lake, sometimes to cover along shore. They have not been seen diving to escape pursuit as diving ducks commonly do.

At Williams Lake, Aug. 2, 1937, when a flock of 80 baldpate, strung out on shallow water along the edge of a marshy bay, were approached from the open lake they became uneasy and swam toward an adjacent part of the shore where willows overhung a low bank. Becoming alarmed more as the result of a closer approach most of them rushed over the water with a great deal of

Cariboo Parklands		
Slough, 1 ac.	Aug. 2, 1937	4
Slough, 20 ac.	" 2, "	10
Williams Lake	" 2, "	81
149 Mile Lake	" 2, "	8
103 Mile Lake	" 4, "	130
105 Mile Lake	" 6, "	7
Elliot Lake	" 7, "	25
Lily Pad Lake	" 7, "	9
Lily Pad Lake	July 6, 1938	6
Westwick Lake	" 9, "	71
Slough, 5 ac.	" 13, "	2
Slough, 2 ac.	" 13, "	5
Elliot Lake	" 19, "	4
Murphy Lake	July 15, 1938	40
Lakes District, Vanderhoof	June 16, 1945	50± including a few females
	" 23, "	23
	July 4, "	80±
	" 15, "	100±
	" 24, "	300±

splashing toward the main lake. A few rose, but with difficulty, and were able to fly only a short distance after which they dropped to the water again. Two birds that were left behind fluttered along the shore for a few yards then climbed the bank and disappeared in the brush.

At 103 Mile Lake, Aug. 4, 1937, approximately 100 eclipse males accompanied a raft of diving ducks. When approached they flapped across the open water at a high rate of speed; none dived as did a few adolescents that were with them. On Aug. 20 the number of flightless males on this lake was reduced to approximately 50 and on Aug. 25 none were seen there.

Food and Feeding Habits

In British Columbia baldpate feed upon the foliage and stems of aquatic plants to a greater extent that does any other pond duck with the possible exception of the gadwall, *Anas streperus.* This fact is reflected in the size of the gizzard and the character of the abrasive material ingested to help macerate the food. It has been observed that a correlation exists between the type of food eaten by different species of pond ducks and the size of the gizzard. Species that eat chiefly bulky foods have larger gizzards in proportion to their size than do those that habitually eat more concentrated foods. Thus the gizzard of the baldpate is large in proportion to the bird's size and weight. The abrasive ingested is almost invariably a fine sand, rarely coarse sand, and never gravel. Gizzards of green-winged teal, mallard, and pintail, species that eat many molluscs and crustaceans, invariably contain relatively large amounts of gravel. The vegetation in the gizzards of baldpates frequently is comminuted and thoroughly mixed with sand so that identification of the plants eaten is difficult or impossible.

On the nesting grounds baldpate obtain much of their food by dipping in the shallows and by surface feeding; on migration they associate with diving ducks and American coots and obtain no small proportion of their daily food by robbing the diving birds of the vegetation they bring to the surface. This habit, considered to be of great importance in the life of the baldpate, is discussed more fully later.

Forest ponds and water holes, which in late summer are completely covered with duckweed, *Lemna minor*, are attractive feeding places and this small plant is eaten extensively.

On the Fraser Delta, baldpate, sometimes in company with other pond ducks, visit cultivated meadows and feed on the fresh green forage plants; clover is particularly attractive to them. When fields are partly flooded, which they sometimes are from November to April, the ducks may concentrate on the flood ponds and their constant feeding, which involves pulling up grass plants by the roots, may result in partial destruction of the crop on these particular areas, so that when waters recede in spring the former flood ponds appear as dark patches among the prevailing green vegetation. During the hunting season feeding is done mostly at night. At times when snow covers the ground baldpate have been seen in rural vegetable gardens feeding on Brussels sprouts or whatever other leafy plants are accessible above the snow. Flocks frequent the golf links on southern Vancouver Island, the extensive lawns of Beacon Hill near Victoria and other similar open territory where green food is available even in the coldest weather. At the last place it is not unusual to see several flocks containing 50 birds and more pasturing the green grass. The smaller isolated islands in Georgia Strait, because of the food to be found there and because comparative safety from hunters is assured, also are attractive to them. Thus Snake Island near Departure Bay, which contains patches of green turf and chickweed between thickets of salal and tall rye grass, is visited frequently.

Along the sea beaches of Vancouver Island and elsewhere, sea lettuce, *Ulva* sp., and other algae are eaten; some baldpate populations appear to live on this food almost exclusively for weeks at a time, avoiding the various small animals such as gastropods and crustaceans, which generally are associated with the algae. On a falling tide the ducks may be seen following the receding waters and pulling up algae as rapidly decreasing depths bring it within their reach.

The commensal relationship with diving ducks and coots referred to earlier in this paper has been noted by several authors who are quoted by Bent (1). When baldpate are associated with a flock of diving ducks they swim quickly from one to another of the birds that have just risen to the surface, and should one have plant material pendent from its bill a baldpate will attempt to snatch it. It is a scene of confused activity as the baldpate weave in and out among the other ducks or make short flights from one section of the flock to another. There are the accompanying noises of constant splashing and the characteristic whistled calls of the males. The ducks from whom food is taken do not resent the robbery by any aggressive action. In addition to the food obtained by this means baldpate also pick up some of the plant foliage that has become detached and risen to the surface, and on the surplus material dropped by the diving birds—representing a considerable amount when a large flock is actively feeding.

The robbery method of obtaining food is more general than the literature on the subject would indicate. In the interior it has been observed as a constant habit not only in winter, when it is necessary to survival because shoal-water feeding grounds are frozen, but in the autumn when baldpate can obtain abundant food in the conventional manner of feeding. Thus at Swan Lake, Okanagan, baldpate habitually associate with coots on relatively deep water and subsist almost entirely on *Chara*, which the coots bring to the surface.

There is evidence also that baldpate accompany, or follow closely, the autumn migrations of diving ducks. For example, at Swan Lake, Okanagan, on Oct. 6, 1943, flocks of baldpate and redhead, flying separately, arrived between 11.30 a.m. and 12.30 p.m.

So also in spring large numbers of baldpate associate with flocks of lesser scaup duck and other diving ducks. In the Lakes District near Vanderhoof flocks up to 30 attended the diving operations of mixed flocks of diving ducks, of coots, and once, May 1, 1945, were observed with a flock of whistling swan, *Cygnus columbianus*, on the Nechaco River.

Postbreeding diving ducks that raft on certain lakes in the Cariboo Parklands are a potent attraction for baldpate. For example, at 103 Mile Lake, Aug. 4, 1937, over 100 males were scattered among a much larger number of lesser scaup duck, canvas-back, and other species. Through the summer similar associations involving smaller numbers are observed frequently. Thus at 103 Mile Lake on Aug. 6, 1940, four adult males, several adult females, and 20 young of different ages accompanied a flock of diving ducks, chiefly lesser scaup duck. At 150 Mile Lake, Aug. 10, 1940, four young baldpate, nearly full-grown, were attached to a band of lesser scaup duck that included both adults and young. At Swan Lake, Okanagan, June 4, 1938, eight baldpate were attached to a flock of 19 redhead and when disturbed all flew off together.

The amount of food obtained by baldpate in summer through association with diving ducks is much less than the amounts obtained in autumn and winter, the reason being that in summer animal food such as amphipods and aquatic insects predominate in the diet of diving ducks and these small objects are swallowed while the feeding duck is submerged. Nevertheless the association persists.

Commensal association begins soon after the young are hatched and it is common to see female baldpate with small young attached to flocks of diving ducks. It has been observed also numerous times that baldpate broods are met with more often on the open water of ponds and lakes than in marsh or brush cover, a habitat preference in marked contrast to the preference of other pond ducks for concealing cover. It is suggested that two characteristic baldpate habits, viz.: commensal association with diving ducks and coots, and preference for an open water habitat, reacting constantly one on the other have developed concurrently to make the species unique among pond ducks in these respects.

The stomach contents of 65 baldpate, comprising 15 adult specimens from the Coast Region, one from Alta Lake, 38 adults, one juvenile, and 10 downy young from the Interior Region, are summarized in the following paragraphs.

Food of Downy Young

The food of 10 downy young consisted of 87.80% animal matter, chiefly insects, and 12.20% vegetation. Three taken at Bradley's Slough, 12 mi. south of Vanderhoof, on July 16, 1945, contained insects in the following percentages of total volume, viz.: 100, 90, 60, the additional food being leech egg cases in one, and *Carex* seeds with vegetable debris in the other. The insects identified were damselfly and dragonfly nymphs, aquatic beetles, both adults and larvae, and midge larvae.

Three from a small slough at Springhouse, Cariboo Parklands, June 20, 1941 – 2, July 7, 1941 – 1, contained 100%, 98%, and 75%, respectively of insect material including mayfly nymphs, aquatic beetle larvae, corixids, and adult and larval midges; a few seeds of *Potamogeton pectinatus* and *Scirpus acutus* also had been eaten.

One collected at Cummings Lake, Cariboo Parklands, June 22, 1943, had eaten damselfly nymphs, corixids, midge adults, and pupae, as well as a few *Sparganium* seeds and leech egg cases, the insect material composing 90% of the total food in the stomach.

One specimen from Williams Lake, Cariboo Parklands, July 4, 1938, contained 90% miscellaneous vegetable matter and 10% insect debris.

Two collected at Tupper Creek, Peace River Parklands, contained respectively 100% and 89% insect material consisting of midge pupae (*Ceratopogonidae*), midge larvae (*Chaoborus* sp.), dragonfly nymphs, corixids, and terrestrial coleopterans. The remaining food items were cladoceran egg cases, one *Carex* seed, and a trace of algae.

Food of Juvenile

The stomach of a juvenile, about one-half grown, collected at 103 Mile Lake, Cariboo Parklands, Aug. 4, 1948, was filled with comminuted grass fibers; two seeds of *Potamogeton pusillus*, and one seed of *Myriophyllum spicatum* together represented less than 1% of the food present.

Food of Adults, Interior Region

The food of 38 adults consisted of 5.75% animal matter, chiefly insects, 29.35% algae, and 64.87% vegetation.

FOOD OF 10 BALDPATE, DOWNY YOUNG; PERCENTAGE OF TOTAL VOLUME

Localities and number of specimens		Crustaceans	Odonata	Corixids	Chironomids	Coleoptera	Misc. insects	Seeds	Misc. vegetation
Bradley's Slough	3	—	26.67	—	15.00	30.00	10.00	5.00	—
Slough Springhouse	3	—	—	9.34	8.33	33.34	40.00	2.33	6.66
Cummings Lake	1	—	20.00	5.00	65.00	—	5.00	5.00	—
Williams Lake	1	—	—	—	—	—	10.00	—	90.00
Tupper Creek	2	5.00	5.00	—	67.00	22.33	—	0.67	—

Animal food 87.80% Vegetable food 12.20%

A specimen from Irish Lake, Sept. 25, 1940, contained comminuted plant foliage exclusively; in another taken at the same place, Oct. 14, 1940, were pieces of a rush, *Juncus* sp., 100%; two seeds of *Carex* sp. and one of *Scirpus acutus* represented less than 1% of the stomach contents.

One from Disputed Lake, Sept. 23, 1940, had eaten several hundred mosquito larvae, *Culex* sp., as well as corixids and 100+ bryozoan statoblasts.

One collected on a small lake near 108 Mile, Oct. 20, 1940, contained 99% miscellaneous plant material including *Lemna minor*, a few seeds of *Scirpus acutus* constituting the remaining 1%.

The three localities referred to are in the Cariboo Parklands.

A specimen from Alta Lake, 37 mi. north of Squamish, Sept. 30, 1944, contained stems and leaves of grass exclusively, and one from the north end of Okanagan Lake, Nov. 17, 1939, was filled with *Elodea canadensis*, the sole item.

The contents of 33 stomachs from Swan Lake, Okanagan, September – 5, October – 22, November – 5, December – 1, are summarized as follows: aquatic vegetation, usually comminuted and mixed with sand, was the sole or major item in 21 stomachs. Plants identified were *Potamogeton pectinatus*, *Potamogeton* sp., *Utricularia* sp., and *Ceratophyllum demersum*. Next in importance were the branches of *Chara* sp., which constituted the exclusive item in seven, and 99% and 94% of the total food in two others. Seeds of *Scirpus acutus* was the sole food in one, 85%, 70%, 50%, 50%, and 25% in five, and represented minor amounts in three other stomachs. Seeds of other aquatic plants eaten were *Potamogeton pectinatus*, *Polygonum Muhlenbergii*, and *Rumex maritimus* (fruits). These seeds were present in small quantities, as were *Chara* oöspores and an unidentified alga. One bird had eaten a bristle-worm, *Nais* sp., another approximately 50 corixids, and a trace of this insect was found in a third specimen.

Food of Adults, Coast Region

Eighteen stomachs of baldpate from the Coast Region represent the following localities, viz.: Sardis, November – 1; Pitt Meadows, December – 1; Nicomen Island, November – 1; Boundary Bay, March – 3; Ione Island,

TABLE III

FREQUENCY OCCURRENCE OF FOOD ITEMS IN 65 BALDPATE STOMACHS

Bryozoa	Statoblasts	1
Bristleworm, *Nais* sp.		1
Leech, Hirudineae	Egg cases	2
Water flea, Cladocera	Egg cases	1
Mayflies, Plectoptera	Nymph	2
Damselflies, Odonata	Nymph	3
Dragonflies, Odonata	Nymph	2
Water boatman, Corixidae	Adult	7
Beetle, terrestrial	Adult	5
Beetle, aquatic	Adult	2
Beetle, aquatic	Larva	2
Midge, Chironomidae	Adult	3
Midge, Chironomidae	Larva	3
Midge, Ceratopogonidae	Pupa	2
Midge, *Caorbornis* sp.	Larva	2
Mosquito, *Culex* sp.	Larva	1
Fly, Diptera	Pupa	1
Ant, *Formica lufa melanotica*	Adult	1
Insect debris, unidentified forms		5
Muskgrass, *Chara* sp.	Branches	9
Muskgrass, *Chara* sp.	Oöspores	2
Algae, unidentified freshwater form		2
Algae, unidentified marine form		2
Algae, sea lettuce, *Ulva* sp.		3
Bur-reed, *Sparganium* sp.	Seeds	2
Pondweeds, *Potamogeton* sp.	Foliage	2
Potamogeton pusillus	Seeds	1
Potamogeton pectinatus	Foliage	2
Potamogeton pectinatus	Seeds	3
Water plantain, *Alisma plantago aquatica*	Seeds	1
Water weed, *Anacharis canadensis*	Foliage	1
Grass, Gramineae	Leaves	12
Grass	Seeds	1
Tall hair grass, *Deschampsia caepitosa*	Seeds	1
Sedge, *Carex* sp.	Seeds	5
Sedge, *Carex Barbareae*	Seeds	1
Sedge, *Carex arcta*	Seeds	1
Bulrush, *Scirpus acutus*	Seeds	11
Scirpus americanus	Seeds	1
Spike rush, *Eleocharis palustris*	Seeds	1
Rush, *Juncus* sp.	Stems	1
Duckweed, *Lemna minor*		1
Dock, *Rumex maritima*	Fruits	1
Water smartweed, *Polygonum Muhlenbergii*	Seeds	1
Water buttercup, *Ranunculus* sp.	Seeds	1
Cinquefoil, *Potentilla montspeliensis*	Seeds	1
Clover, *Trifolium* sp.	Leaves	1
Water milfoil, *Myriophyllum spicatum*	Seeds	1
Hornwort, *Ceratophyllum demersum*	Foliage	1
Bladderwort, *Utricularia* sp.	Foliage	1
Unidentified plant debris	Foliage, stems	20

November – 1; Lulu Island, November – 1; Albert Head, January – 1; Nanaimo Flats, December – 1; Departure Bay, December – 1; Snake Island, near Departure Bay, January – 1; Quennell Lake, January – 1; Comox, March – 2. The six localities last named are on Vancouver Island.

One specimen from each of the localities Albert Head, Nanaimo Flats, and Quennell Lake contained grass leaves and fibers exclusively. The sole item

TABLE IV

FOOD OF 54 ADULT BALDPATE, PERCENTAGE OF TOTAL VOLUME

Locality and number of specimens	Insects, other invertebrates	Chara	Other algae	*Scirpus* seeds	Other seeds	Grass	Foliage aquatic plants
108 Mile Ranch 1	—	—	—	1.00	—	—	99.00
Irish Lake 2	—	—	—	—	—	—	100.00
Disputed Lake 1	100.00	—	—	—	—	—	—
Okanagan Lake 1	—	—	—	—	—	—	100.00
Swan Lake, Okanagan 33	1.16	27.19	1.07	10.25	1.39	3.03	55.91
Alta Lake 1	—	—	—	—	—	100.00	—
Boundary Bay 3	—	—	—	—	—	100.00	—
Sardis 1	—	—	—	—	50.00	50.00	—
Nicomen Island 1	2.00	—	—	—	1.00	—	97.00
Ione Island 1	—	—	—	—	—	100.00	—
Lulu Island 1	—	—	100.00	—	—	—	—
Pitt Meadows 1	1.00	—	—	—	99.00	—	—
Nanaimo Flats 1	—	—	—	—	—	100.00	—
Departure Bay 1	—	—	100.00	—	—	—	—
Snake Island 1	—	—	100.00	—	—	—	—
Quennell Lake 1	—	—	—	—	—	100.00	—
Comox 2	—	—	100.00	—	—	—	—
Albert Head 1	—	—	100.00	—	—	—	—

in two from Comox and one from Departure Bay was sea lettuce, *Ulva* sp., and one from Snake Island contained an unidentified marine alga only.

Three collected in a hay field at Boundary Bay, Mar. 24, 1922, had eaten new grass shoots some with roots attached, and one stomach contained a few clover leaves, *Trifolium* sp.

The stomach of a specimen from Sardis, Nov. 20, 1934, held equal amounts of grass leaves and seeds of *Sparganium* sp.

Grass fragments represented 99% of the contents of one from Nicomen Island, in the Lower Fraser River, Nov. 4, 1934, the remaining small quantity of food present being *Eleocharis palustris* seeds and corixid debris.

One specimen from Ione Island in the mouth of the Fraser River, Dec. 29, 1941, contained grass leaves and four seeds of *Scirpus americanus.*

The sole food item in one from Lulu Island, Nov. 10, 1946, was a marine alga.

Seeds of aquatic plants constituted 99% of the food in a specimen from Pitt Meadows, Dec. 16, 1923, the species represented being: sedge, *Carex Barbareae* and *C. arcta,* hair grass, *Deschampsia caespitosa,* and cinquefoil, *Potentilla monspeliensis.* Eight pupae of a fly and the head of a terrestrial beetle composed the remaining one per cent.

Food Summary

The food of 10 downy young collected in the Cariboo Parklands, the Peace River Parklands, and the Vanderhoof Region was composed chiefly of aquatic insects, the most prominent items being chironomid larvae and Odonata nymphs. Miscellaneous vegetation was the chief food eaten by one half-grown young, and the seeds of aquatic plants were present in three stomachs. In the diet of adults the foliage of aquatic plants was first, grasses second, and algae third in times of occurrence and percentage volume. Seeds of aquatic plants were less often represented, and animal matter, chiefly insects, was of minor importance, the total percentage volume being only 5.75%.

Economic Status

In the winter and spring baldpate cause damage to agricultural crops in the Lower Fraser Valley by concentrating on shallow flood ponds that have formed in the fields and by feeding on young forage plants growing there. Damage to fall-sown grains also occurs when the young plants are small. The habit is a local one, the damage caused is not usually serious and is amenable to control. Damage to vegetable crops, particularly lettuce, so common a cause for complaint in the southwestern United States, and providing a problem in game management that so far has not been resolved, is of slight importance in British Columbia. The value of the species as a game bird, and for food, would seem to more than balance the loss suffered by agriculture in British Columbia. This duck does not eat salmon eggs or the flesh of spent salmon, and rarely if ever eats marine crustaceans or molluscs – foods that taint the flesh and that are commonly eaten by mallards and some other duck species wintering on the coast. Consequently the baldpate is esteemed as a table bird.

Acknowledgments

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ON THE ANATOMY OF *GRYLLOBLATTA CAMPODEIFORMIS* WALKER

5. THE ORGANS OF DIGESTION[1]

BY E. M. WALKER[2]

Abstract

The digestive organs of *Grylloblatta* resemble those of the orthopteran suborder Ensifera but differ strikingly in the cuticle and epithelium lining the proventriculus, in which there are 12 similar longitudinal divisions characterized by rows of flexible, backwardly directed lamellae, in place of the six divisions of the Ensifera armed with columns of complex sclerotized teeth. The proventricular collum is much longer than in any of the Ensifera; the two gastric caeca of the latter are represented by a single bilobed sac; the malpighian vessels are fewer than 30 and are simply arranged; and the salivary glands are very compact, appearing like a single organ.

As a main conclusion to this and the previous papers of this series the following views are advanced:

(1) The Grylloblattaria, although cursorial, are the nearest relatives of the Ensifera, but differ from the latter group too widely to be included within it.

(2) The saltatorial habit has been independently evolved in the Ensifera and Caelifera.

A summary is given of the main views on the function of the proventriculus in mandibulate insects, particularly the orthopteroid forms. The proventriculus of *Grylloblatta* is believed to serve as a regulatory valve and also possibly as a propulsive organ for the movement of food along the digestive tract.

Introduction

The present study of the digestive organs of *Grylloblatta* is based upon even scantier material than were the previous parts of this series, viz., a few dissections chiefly of adult individuals and a single series of sections of a male nymph, about 10 mm. long, which was made many years ago by my wife.* This specimen was fixed in Carnoy's fluid, embedded in paraffin, and cut into sections 15μ in thickness. Two combinations of stains were used, Heidenhain's iron haematoxylin with orange G for about three-fifths of the series, and Delafield's haematoxylin with eosin for the remainder. The former stain gave slightly the better results of the two.

Much has been written on the digestive organs of insects, and the orthopteroid orders, being relatively primitive groups, have received an ample share

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of attention. Following Dufour's (20) general survey of the anatomy of the old Order Orthoptera (together with Hymenoptera and Neuroptera), the next important general treatise on the anatomy of the digestive organs of these insects is that of Bordas (12). This is still a useful general survey of the gross anatomy of the alimentary canal and its appendages in the main groups of orthopteroid insects, treated from the comparative standpoint. Bordas also dealt with some of the families in greater detail, viz., the Forficulidae (5), Gryllidae (8, 9, 12), Phasmidae (7), Acrididae (10), and Blattidae (6, 9), besides publishing many papers on special features of the anatomy and histology of the alimentary canal and its associated glands.

Numerous papers by other authors on the anatomy and physiology of the digestive system of insects appeared in the last quarter of the nineteenth century, including a considerable number on the function of the proventriculus, in which opposing views were presented. Reference will be made later to some of these when the function of the proventriculus of *Grylloblatta* is considered.

During the twentieth century the digestive organs of the Isoptera have become well known through the writings of Imms (31), Holmgren (30), and Sutherland (49), and the inner structure of the proventriculus has been recently described by Judd (33) in the genus *Termopsis*. While possessed of certain peculiar features associated with their xylophagous habits, the alimentary canal in this order is sufficiently like that of the Blattodea to be in accord with the now generally accepted view of the Isoptera as an order of orthopteroid insects.

Until the third decade of the present century two small families of Saltatoria, viz., Tridactylidae and Cylindrachetidae, were generally placed by taxonomists in the Grylloidea, owing to their superficial resemblance to the Gryllotalpidae. Dufour (20) had already recognized that the affinities of *Tridactylus* were with the Acridoidea, particularly the genus Tetrix, basing his view on the characters of the alimentary canal and the reproductive organs. Walker (52) noted the similarity between the ovipositors of *Rhipipteryz,* another genus of Tridactylidae, and the Acridoidea. But the majority of taxonomists continued to treat these families as specialized Gryllidae or at least Ensifera, until the detailed studies of Carpentier (14, 15, 16, 17), Ander (1), and Fernando (24) appeared. These studies, in which the alimentary canal of both *Tridactylus* and *Cylindroryctes* are described, present evidence amounting virtually to proof, of the view that these two families are nearly related to one another, and belong to the same suborder as the Acridoidea, this suborder being now known as the Caelifera. The recent comparative studies of the proventriculus of orthopteroid insects by Judd (33) add still further evidence of the soundness of these conclusions.

Other important advances in orthopterology have been made during this century, which throw light on the phylogeny of the major groups through our increasing knowledge of the fossil record, as well as through more critical study of the anatomy of living forms. Handlirsch's (26) monumental work on fossil insects remains the foundation for all future studies in this field. Zeuner (60) in his exhaustive treatise on the fossil Ensifera has traced this group back to certain protorthopteran families of the Upper Carboniferous period and believes that the Acridoidea and their nearest relatives (later termed the Caelifera by Ander) were derived by a separate line of descent from nearly related protorthopteran families of the same period. Ander (2, 3) approaching the problem from the standpoint of comparative anatomy of recent Ensifera, supports Zeuner's view that the Ensifera and Caelifera represent distinct lines of descent at least as far back as the Upper Carboniferous and emphasizes the impossibility of deriving the Caelifera from the Ensifera. His main objective, however, is to determine the interrelationships of the various families of Ensifera, and in this phase of the subject his views differ considerably from those of Zeuner. They are, however, of significance here only in so far as it is possible to determine what are the primitive characteristics of the Ensifera. Ander's scholarly study is the most important work from our point of view that has yet appeared, since it is based not merely upon one or two sets of characters, but upon a consideration of the anatomy as a whole.

It may be added that, during the present century, a number of comprehensive textbooks have appeared in which useful summaries of the structure of the alimentary canal are given. Among these may be specially mentioned Berlese (4), Imms (32), Deegener (19), Weber (57), and Snodgrass (48). We have found Snodgrass the most generally useful for the purpose of the present paper, and the terminology employed therein has been followed as closely as possible.

The Preoral Cavity

The space usually known as the "mouth cavity" or "buccal cavity" has been more correctly designated as the *preoral cavity* by Snodgrass (48), since it lies wholly external to the true mouth. It is the space enclosed by the mouth parts, being bounded in front (or above) by the labrum, behind (or below) by the labium, and laterally by the mandibles and maxillae. These parts have been already described for *Grylloblatta* in Part 1 of this series (Walker, 53).

Since *Grylloblatta* is a prognathous insect, the preoral cavity opens (by the food meatus, Snodgrass (48)) at the anterior extremity of the ventral surface of the head. The epipharyngeal surface of the labrum and clypeus form its roof rather than its anterior wall (as in most Orthoptera), while the labium forms its floor rather than its posterior wall (Fig. 2). Apart from these differences from the majority of Orthoptera in its relations to the horizontal axis of the head, the preoral cavity of *Grylloblatta* conforms to Snodgrass's description as applied to Orthoptera in general. It is bounded laterally by the mandibles and maxillae, which are directed forward, and distally curved somewhat ventrad. It is divided by the hypopharynx (Figs. 1, 2, *hy*) into a salivary chamber below, and a food passage above, the latter space opening immediately behind the labrum and between the apical parts of the mandibles in the food meatus.

The salivary chamber or *Salivarium* (Figs. 1, 2, *sv*) is a simple pocket between the hypopharynx and the prementum of the labium (*pm*). There is no salivary cup, the orifice of the salivary duct being a simple median aperture at the posterior limit of the pocket.

The food passage leads from the food meatus to the *Cibarium* (Figs. 1,2, *cb*), which is floored by the concave upper surface of the basal part of the hypopharynx, and is roofed by the epipharyngeal surface of the clypeus.

A pair of muscles, the *dilators of the cibarium* (Fig. 2, *dcb*) were overlooked in Part 1 of this series. These muscles arise in the base of the dorsal wall of the clypeus and pass anteroventrad to their insertion on the dorsal wall of the cibarium.

Another muscle overlooked in Part 1 is the single median *compressor of the labrum* (Fig. 2, *clr*), which arises on a small area on the base of the dorsal wall of the labrum, its fibers diverging ventrad to their insertion on the epipharyngeal wall of the labrum.

At the base of the hypopharynx, flanked by the bases of the mandibles, is the true *mouth* (*m*), from which the alimentary canal extends backwards.

The Labial or Salivary Glands and Their Ducts (Figs. 1-5)

The salivary orifice already mentioned is the opening of the median or common duct of the labial glands (Figs. 1–3, *msd*), which in *Grylloblatta* is a short, somewhat dilated chamber lying immediately above the mentum of the labium, and apparently functioning as a salivary reservoir, there being no other chamber which could serve this purpose. From this common outlet two slender *salivary ducts* (*sd*) proceed backwards from the region of the mentum into that of the submentum. Here they lie immediately beneath the suboesophageal ganglion, but, on entering the cervical region, they pass dorsally to the ventral nerve cords, as the latter leave the ganglion and take up a position just beneath the oesophagus to which they are closely applied, From here backwards they diverge and, in sections through the prothorax. may be observed close to the lateral walls of the oesophagus, passing gradually dorsad until they enter the substance of the labial glands (Fig. 4).

The usually paired *labial glands* (*sgl*) are, in *Grylloblatta*, so closely applied to one another that they appear throughout the greater part of their length as a single compact glandular mass lying on the dorsal wall of the oesophagus in the prothorax (Figs. 1, 4). Approaching the mesothorax this glandular mass sinks ventrad on the sides of the oesophagus (Fig. 5), to which it is closely applied, and still farther back in the mesothorax these ventrolateral extensions meet below, the oesophagus being for a short distance completely surrounded by the glandular tissue. Towards the posterior limit of the mesothorax, only the ventral parts of the gland remain, and here the right and left portions are narrowly separated.

The ducts of the labial glands, entering the substance of the glands, break up into numerous canaliculi surrounded by the somewhat irregular acini (Fig. 5). The cells of the acini are large, with basally situated nuclei, which are somewhat flattened or irregular in form, and very deeply stained, no details of structure being visible in the sections. The cytoplasm is irregularly stained, the accumulations of secretion giving it a more or less vacuolated appearance towards the inner borders of the cells. In many cases drops of secretion may be seen entering the canaliculi.

The Alimentary Canal (Fig. 8)

The alimentary canal may be described under its three primary divisions, the stomodaeum, mesenteron, and proctodaeum.

The Stomodaeum

The *stomodaeum* consists of five divisions, the buccal cavity, the pharynx, the oesophagus, the crop or ingluvies, and the proventriculus.

The *buccal cavity* (Fig. 1) is a very short section of the stomodaeum, having a slight dorsoposterior curvature, as it leads to the pharynx, from which it does not differ in structure. It is that portion of the stomodaeum on which are inserted the first pair of dilator muscles termed in Part 1 (Walker, 53) the *dorsal dilators* of the buccal cavity (Muscle No. 3. See Part 1, Plate III, Fig. 27). These muscles in *Grylloblatta* arise on the base of the labrum instead of the clypeal wall, the latter being the usual site of attachment in generalized insects according to Snodgrass (48). They are omitted in Fig. 2 of the present paper. The buccal cavity passes into the pharynx at the anterior end of the frontal ganglion.

The *pharynx* as defined by Snodgrass (48) is "that part of the stomodaeum whose dorsal dilator muscles take their origin on the frontal and dorsal areas of the head wall, and are inserted posterior to the frontal ganglion and its connections". This definition applies to *Grylloblatta*, which possesses three pairs of dorsal dilators, except in so far as the first two pairs of dilators (Walker (53), Pl. III, Fig. 27) are inserted laterad of the frontal ganglion, rather than behind it. The third pair (8) is inserted behind the cerebral ganglion, and this marks the posterior boundary of the pharynx, since the second of the two pairs of ventral dilators is inserted opposite the third dorsal pair (Walker, 53).

The pharynx extends directly backwards from the buccal cavity, which it leaves at a slight bend (Fig. 2, *ph*). It is the smallest in caliber of any part of the alimentary canal. It is lined by a thin cuticle supported by a somewhat flattened epithelium, which is extensively and rather irregularly folded.

External to the basement membrane is a thin muscular layer, the inner fibers of which are longitudinal and discontinuous, the outer fibers circular and more close set. The thickness of each layer is that of a single fiber.

The *oesophagus* (Fig. 1, 2, 4, 5, *oe*) as it proceeds from the pharynx is but little larger in diameter, and scarcely widens as it passes through the prothorax. Throughout most of this region, as already described, the oesophagus is covered by the labial glands, which, in the anterior part of the mesothorax, surround it for a short distance and terminate beneath it. In most of the specimens dissected, the oesophagus widens only very gradually until the metathorax is entered, but the extent of dilatation depends upon the volume of its contents.

The *crop* or *ingluvies* (Figs. 1–7, *cr*) is merely the expanded posterior part of the oesophagus, the expansion being mainly ventral, as in the Grylloidea, with the widest part near the posterior end, at about the third or fourth abdominal segment, where it is somewhat abruptly rounded off (Fig. 7).

The walls of the oesophagus and crop (Fig. 6) are similar throughout their combined length. The epithelium is very flat, and is longitudinally folded when not distended with food. No histological differences were found between the oesophagus and pharynx, except in the flatter epithelium of the former.

The *proventriculus* (Figs. 1, 7, 9, 10) has been described by Judd (35). It is well developed and appears externally very like that of the saltatorial suborder Ensifera, although longer than is usual in this group. It is a thick-walled muscular tube, composed of an anterior necklike region or *collum* and a posterior bulbous part, which may be termed the *bulb*. It leaves the crop dextrodorsally a little before the posterior end of the latter. The first part or collum is bent somewhat to the right and folded into an anterodextral loop, the posterior limb widening into the bulb, which lies in a depression in the roof of the ventriculus near the anterior end of that chamber.

In the collum, the lining epithelium and cuticle are arranged in 12 longitudinal folds (*a*), which are comparatively flat as they approach the bulb, but become prominent as they enter its cavity. The cuticle covering these folds is beset with minute hairlike processes, rather evenly distributed and all directed backwards.

In the bulb these 12 folds (Fig. 9) are narrowed to pointed apices and in the angular intervals between these apices an alternating series of folds (*b*) arises, which form the stout bases of 12 wedge-shaped processes (*c*), each of which is demarcated from its base by a distinct notch. Alternating with these 12 processes, and therefore in alignment with the 12 anterior folds, is a second series of processes, which are longer and more compressed than those of the first series and project into the cavity of the proventriculus a little behind the first series. These are very thin and lamellate except towards their free extremities where they are somewhat swollen. The 12 folds of this second series are again subdivided into two alternating sets of six each, differing in length and in the form of their free extremities, those of one set (*d*) being longer, extending farther back, and having sharper apices than those of the other set (*e*). These various folds or processes are thus arranged in three overlapping rows, the first row consisting of the 12 wedge-shaped processes, overlapping the bases of the second row of six flattened processes or lamellae and this second row overlapping the third row, consisting of six longer lamellae. All of the processes are beset with fine hairs, which are longest and most abundant along their free margins and at their apices.

The circular muscles of the proventriculus form a much thicker layer than those of the other divisions of the stomodaeum (Fig. 9, 10, *cm*). The longitudinal muscles internal to the circular layer are comparatively thin, although thicker than those of the oesophagus and are broken into bundles which occupy the folds. In the bulb there are also many scattered longitudinal muscle fibers which lie external to the circular layer, including isolated fibers which pass through the haemocoele to the outer surface of the ventriculus, (Fig. 7).

The manner in which the proventriculus probably functions will be considered in the general discussion (p. 331).

The *stomodaeal valve* (Fig 10, *sti*) is the last division of the stomodaeum and is that part which projects as a circular fold into the cavity of the ventriculus and is usually termed the cardiac valve. It is rather short in *Grylloblatta* (Fig 10). The smooth cuticle which covers it can, as usual, be traced over the fold to its junction with the ventricular wall.

The Mesenteron

The mesenteron consists of a single section of the gut commonly termed the *ventriculus* or stomach. It occupies the middle region of the abdomen, being usually found in the fourth and fifth or the fifth and sixth segments. In its natural position (Fig. 1) it is bent more or less ventrad upon its junction with the proventriculus. It is a short wide sac, broadest towards its anterior end. Here there is a dorsal concavity (Fig. 7) which receives the bulb of the proventriculus, and on each side and below the depression there is a forward bulging of the wall forming a pouch which is slightly bilobed, representing an incipient pair of gastric caeca (*gc*). The ventriculus is slightly twisted so that the right caecum lies a little higher than the left. Posteriorly the ventriculus is somewhat narrowed to its junction with the intestine.

The ventricular epithelium (Figs. 10, 11) is similar throughout its extent, not being specialized in the gastric caeca. It is arranged in tall columnar folds, composed of digestive cells with groups of smaller regenerative cells at their bases. The regenerative cells, being crowded by mutual pressure, show flattened or crescentic nuclei, which are well stained, and small cytoplasmic areas. Indications of mitosis may be observed here and there. As the cells migrate toward the lumen, becoming the digestive cells, they are more elongate with elliptical nuclei, which stain more faintly the nearer they approach the lumen. The cytoplasm is more abundant and is finely granular in appearance. There is no trace of a peritrophic membrane, a fact that is not wholly surprising in view of the apparent absence of hard particles in the food.

The epithelium in the specimen sectioned was in a state of active disintegration involving the separation of numerous fragments of cells. There is thus no indication of a striated border. The ventricular cavity is open and must have contained fluid material but there is no definite indication of the presence of solid particles.

External to the basement membrane is a thin but continuous layer of circular muscle fibers, followed by a more scattered layer of longitudinal fibers, some of which pass forward to the proventriculus as already described.

THE PROCTODAEUM OR INTESTINE

The intestine of *Grylloblatta* is of moderate length and follows a somewhat sinuous course with a double curvature (Fig. 1). Its two main divisions, termed by Snodgrass the anterior and posterior intestines, are sharply distinct.

At the anterior end of the intestine is a constriction which appears externally as the junction between the ventriculus and intestine. A slight thickening of the circular muscles at this constriction forms a small pyloric sphincter (Fig. 12).

The *malpighian vessels* (Figs. 8, 12, 13) open into the intestine at the pyloric constriction, just behind the sphincter muscle. They evidently vary in number, one adult female in which they were carefully counted having had 17, another 14, a third about 24, while in the series of sections made from a male nymph there appear to be only 12. They are fairly evenly distributed and close set at their junctions with the intestine. They are unbranched and of considerable length, winding both forwards and backwards throughout most of the abdominal cavity. In thickness they are nearly uniform in adults but in sections of the nymph many are considerably enlarged distally (Fig. 13).

The *anterior intestine* (Figs. 1, 8, 12, 13) is short and not divisible into ileum and colon. It generally appears to be much smaller in diameter than the ventriculus and becomes still smaller as it approaches the beginning of the posterior intestine. As it leaves the ventriculus it has a ventral position but follows a curved course to the left and then dorsad, taking another bend backwards just before joining the posterior intestine.

At the anterior end, in the serial sections, it appears in cross section (Fig. 13) and here the lining epithelium is thrown fairly symmetrically into six longitudinal folds, which continue throughout the course of the anterior intestine. Examination of the lumen in dissected specimens shows that these folds of epithelium originate just in front of the constriction, there being thus a very rudimentary pylorus or pyloric region.

The epithelium of the anterior intestine consists of columnar cells, regularly arranged, with elliptical nuclei halfway between base and apex. The cytoplasm is clear and is most dense at the inner ends, where a striated border can be seen. A thin but distinct cuticular intima covers the inner surface of the epithelium. As already noted there is a slight transverse fold of the epithelial layer at its junction with the ventricular epithelium, just in front of the pyloric constriction.

The muscularis is very thin and consists, as usual, of an inner continuous circular layer and outer longitudinal fibers which are sparse and somewhat scattered.

The *posterior intestine or rectum* (Figs. 1, 8, 15).—A distinct constriction separates the anterior and posterior intestines, which is followed by a marked increase in the diameter of the gut. This constriction marks the position of the rectal valve where the columnar epithelium of the anterior intestine forms a short but distinct backward fold before joining the flatter epithelium of the rectum. The rectum follows a straight course to the anus and is somewhat indistinctly divided into a wider anterior portion, the *rectal sac*, and a narrower posterior region, the *rectum* proper. The epithelium lining both of these regions is for the most part extremely flat and is covered by a distinct cuticle. In the rectal sac, however, there are six very prominent *rectal pads* (Figs. 1, 8, 15), about equally spaced and arranged in a partially doubled transverse series, two or three pads being in advance of the others. Each pad is broadly oval in outline, with the inner surfaces flattened, and is composed of a single layer of tall columnar epithelial cells with their oval nuclei in the inner half and the cytoplasm most dense next to the inner borders. The muscularis is thinner than usual in orthopteroid insects. The circular muscles form a thin but continuous coat, especially on the rectal sac. The longitudinal muscles consist, on the rectal sac, chiefly of six equally spaced bundles, which extend posteriorly to the anus, but on the walls of the rectum proper there are numerous, somewhat smaller bundles in the intervals between the six main bundles.

Special rectal muscles.—Owing to an insufficiency of material for satisfactory dissection, the following descriptions of muscles are subject to revision:

Retractors of the rectum (Fig. 8, *rtr*). A pair of slender bundles of parallel fibers arising on the anterior margins of the ninth tergum near its anteroventral angles, and passing mesocaudad to the lateral walls of the rectum, where they join the longitudinal fibers of the muscularis continuing to their insertion on the rectal wall close to the anus.

Dorsal dilators of the rectum (Fig. 8, *ddr*). A pair of thin fans of slender fibers arising on the anterior margins of the ninth tergum internal to the retractors of the rectum, diverging mesad very widely to their insertion on the dorsal wall of the rectum partly on the rectal sac but chiefly on the rectum proper. These fibers are supported by a thin membranous sheath.

Ventral dilators of the rectum (Fig. 8, *vdr*). A pair of small bundles of fibers arising on the ventrolateral part of the 10th tergum and passing mesad to their insertion on the ventral wall of the rectum proper.

Dorsal dilator of the anus (Fig. 8, *dda*). A short median unpaired muscle arising centrally on the 10th tergum and passing ventrad to its insertion on the dorsal wall of the rectum close to the anus.

Ventral dilators of the anus (Fig. 8, *vda*). A pair of small muscles arising on the paraproct and passing mesad to their insertion on the lateral walls of the rectum close to the anus.

Comparison with Other Orthopteroid Insects

The gross general characteristics of the digestive organs of orthopteroid insects are the following:—

The foregut is divided into buccal cavity, pharynx, oesophagus, crop, and proventriculus. The oesophagus passes from the head into the thorax and widens there into a more or less spacious crop generally extending throughout the greater part of the thoracic length, often well into the abdomen. The proventriculus is usually a distinct chamber with thickened muscular walls and a specialized cuticular lining, but may be little more than the stomodaeal or cardiac valve, and may be indistinguishable in external view.

The mid-gut is a cylindrical tube, usually shorter than the fore-gut, generally straight or nearly so, and of about uniform caliber, more rarely curved or even forming a complete loop. At the anterior end its wall is generally produced into two or more diverticula, the *gastric caeca,* but these are not always present. The mid-gut terminates in a slight valvular constriction, the pyloric valve, and is followed by the intestine, which is divided into two regions, the anterior and posterior intestines. The anterior intestine may be straight and axial or it may exhibit various degrees of curvature, being sometimes distinctly convoluted. It is often divided into two regions of different caliber, the more dilated part being sometimes anterior, sometimes posterior, or there may be three divisions, the dilated part being the middle one. The hind intestine, commonly known as the rectum, is generally an ovoid chamber and contains six epithelial cushions, the rectal pads or rectal glands. Malphigian vessels are always present, opening into the anterior intestine immediately behind the pyloric valve. They vary greatly in number and arrangement, being sometimes distributed uniformly, sometimes in groups, each group with a common duct, or, in extreme cases, there may be a single common duct for all of the vessels.

The salivary or labial glands in orthopteroid insects are much divided, paired structures, lying in the thorax chiefly in the region of the oesophagus, the ducts from each glandular mass uniting below the digestive tube to form a common duct, which opens on the ventral wall of the head between the labium and the hypopharynx. In most forms there are paired salivary reservoirs, which are large diverticula of the salivary ducts, usually the paired ducts, but sometimes of the common duct.

In this section the digestive organs of *Grylloblatta* are compared with published descriptions of these organs in the following orthopteroid groups:—

Order	*Family*
Dermaptera	Forficulidae
Plecoptera	Pteronarcidae, Perlidae, Nemouridae
Blattodea	Blattidae
Mantodea	Mantidae
Isoptera	Hodotermitidae, Termitidae
Phasmida	Phasmidae
Saltatoria	
Suborder Caelifera	Acrididae, Tetrigidae, Tridactylidae, Cylindrachetidae
Suborder Ensifera	Tettigoniidae, Gryllacrididae, Rhaphidophoridae, Stenopelmatidae, Gryllidae, Gryllotalpidae.

DERMAPTERA

In general plan the digestive tube of *Grylloblatta* is not unlike that of *Forficula auricularia* L. as described and figured by Bordas (5).

Features common to these forms are:

(1) The course of the tube is for the most part straight, having only a slight to moderate convolution in the region of the stomach and intestine.

(2) The external divisions are the same, viz., *fore-gut*—pharynx, oesophagus, crop, proventriculus; *mid-gut*—ventriculus or stomach; *hind-gut*—anterior intestine, posterior intestine or rectum.

(3) The relatively short hind-gut, of which the anterior part (anterior intestine) is not divided into ileum and colon.

(4) The somewhat feeble cuticular armature of the proventriculus, which in neither form could be used for triturating the food.

(5) The relatively small number of malpighian vessels (smaller in *Forficula*).

(6) The alternate arrangement of the six rectal pads.

The differences are more striking. The more important of these may be listed as follows:

(1) The relatively small crop in *Forficula*, which according to Bordas' figure, is smaller than the mid-gut, whereas in *Grylloblatta* it is very much larger.

(2) Differences in the proventriculus:— absence in *Forficula* of the collum which is unusually well developed in *Grylloblatta*; internal lamellae in *Forficula* six instead of 12 as in *Grylloblatta*, their forward projections into the terminal part of the crop and their backward extensions into the mid-gut in the form of six long valvular processes having no counterpart in *Grylloblatta*.

(3) The much larger ventriculus in *Forficula*, with *no trace of gastric caeca.*

(4) The grouping of the malpighian vessels in *Forficula* into two fascicles, in contrast to their even distribution around the pylorus in *Grylloblatta*.

PLECOPTERA

In the few genera of stone-flies in which the digestive organs have been studied there appear to be wide variations in several directions.

In the primitive genus *Pteronarcys*, according to Newport (38), the oesophagus gradually widens into a crop that extends through the thorax into the abdomen as far as the fourth segment. Although Newport makes no mention of a proventriculus and there is no indication of one in his figure, it is probably developed to some degree in the nymph. There are no gastric caeca but the ventriculus is remarkably long and convoluted, extending posteriorly to the ninth abdominal segment, where it meets the extremely short intestine. The latter is divided into two sections designated ileum and colon by Newport, but doubtless the anterior and posterior intestine, according to our terminology. The malpighian vessels number 40 to 50 and appear in Newport's figure to be evenly distributed around the pylorus.

Nemoura vallicularia, investigated by Wu (59), and *Perla* by Schoenemund (47) and Imhof,* both differ from *Pteronarcys* in the straight course of the alimentary canal. *Nemoura* resembles *Pteronarcys* in the lack of gastric caeca and in the very long ventriculus, which extends from the prothorax to the end of the fourth abdominal segment. It is unlike *Pteronarcys* in the very short stomodaeum, which terminates in the prothorax, instead of passing halfway through the abdomen. The expanded posterior part, termed by Wu the proventriculus, is evidently the crop.

In *Perla*, there is again a very large crop, extending well into the abdomen. The nymphal proventriculus has been studied by Schoenemund (47) and that of the related genus *Acroneuria* by Judd (33). It is cylindrical and but slightly marked off externally from the crop. The inner lining in both of these genera is unlike that of most orthopteroid insects but resembles that of *Grylloblatta* slightly in that there are, over a part of its area, 12 similar divisions, i.e., longitudinal folds or ridges, instead of six, which is the usual number in orthopteroid insects. The ventriculus is much shorter than in *Pteronarcys* and *Nemoura* but there is a circle of forwardly directed gastric caeca, which are slender and conical like those of the Acridoidea. There are eight of these according to Schoenemund, 10 according to Imhof, and seven in Acroneuria (Judd, 33). In all of these forms there are two lateral caeca which are longer than the others. The gastric caeca are present in the adult stage as well as the nymph (Schoenemund, 47).

The intestine is short in all of the plecopteran genera described and in each form it pursues a straight course. The anterior intestine ("colon") of *Perla* sp., is described by Schoenemund as opening into the rectum somewhat asymmetrically, and in the same genus (according to the generic concept of the period), Newport (38) describes and figures a distinct caecum arising from the rectum ("colon"). The malpighian vessels vary from 36 in Nemoura to about 80 in *Perla*. They appear to be arranged usually in a simple circle in the forms described.

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* *According to Imms (32) and Weber (57).*

Grylloblatta resembles all the plecopteran genera described here in the division of the intestine into two regions only, and in the simple arrangement of the malpighian vessels. It is like *Pteronarcys* and *Perla* in the long oesophagus and crop, extending into the abdomen, and it is somewhat like *Perla* in the short wide ventriculus. It also resembles *Perla* and *Acroneuria* slightly in the inner structures of the proventriculus, the lining epithelium and cuticle, over a part of its area, being raised into 12 similar longitudinal folds or ridges instead of the usual six.

Grylloblatta differs from the Plecoptera in the form of its very muscular proventriculus with collum and bulb, in the number and very different form of the gastric caeca and in the longer and somewhat convolute intestine. In general the alimentary canal of the Plecoptera is more like that of the Caelifera than that of *Grylloblatta*.

BLATTODEA

The organs of digestion in the cockroaches have been described by a number of authors, e.g. Miall and Denny (37), Bordas (6, 9), and Petrunkewitsch (39). Most of the work, however, has been done on the two common and nearly related forms *Blatta orientalis* and *Periplaneta americana*, but Bordas gives general descriptions of a number of genera. In these there is considerable variation, but the common plan of structure is the same.

With the Blattodea *Grylloblatta* shares the general features of the digestive system as already outlined, including a thick-walled proventriculus visible in external view, a mid-gut with at least the beginnings of gastric caeca, and an intestine that has at least one flexure. *Grylloblatta*, however, differs strikingly from the Blattodea in certain respects, viz.:

(1) The presence of a muscular tube or collum between the crop and the bulb of the proventriculus.

(2) The very different structure of the cuticular intima of the proventriculus the parts of which are arranged on a plan of 12 similar divisions instead of six and are quite different in detail from those of any of the Blattodea, so far as known (*vide* Judd, 33).

(3) The much shorter mid-gut with the broad incipient pair of gastric caeca, instead of the eight slender tubules of the Blattodea.

(4) The much shorter hind-gut of which the first section or "anterior intestine" has no subdivisions such as are found in the Blattodea,

(5) The fewer malpighian vessels, arranged in a circle, with independent openings, in place of the numerous tubules in the Blattodea divided into six groups, each having an opening into a conical elevation of the intestinal wall.

(6) The more compact salivary glands whose main ducts lack the usual salivary reservoirs, which are present in the Blattodea.

MANTODEA

In the digestive system as in all the organ systems the Mantodea give evidence of near relationship to the Blattodea. The chief differences lie in the greatly elongated oesophagus, the inner structure of the proventriculus (Judd, 33), the shorter mid-gut, the usually shorter and less convoluted hind-gut, the anterior intestine not being subdivided as in the Blattodea, and the simple arrangement of the malpighian vessels, which are not grouped, each having its independent opening into the anterior end of the intestine.

Grylloblatta resembles the Mantodea in the same features as indicated for the Blattodea and, further, in the shorter mid-gut, less convoluted anterior intestine, lacking division into two distinct regions, and the simple arrangement of the malpighian vessels, which however, are fewer than in the Mantodea. The greater resemblance of *Grylloblatta* to the Mantodea than to the Blattodea, however, has probably no phylogenetic significance.

ISOPTERA

The digestive organs of the termites have been investigated by Holmgren (30), Imms (31), Sutherland (49), and others, and a good general account of them is given by Imms (32). The organs are not unlike those of the Blattodea, particularly in the more primitive genera (Hodotermitidae). This similarity is seen, e.g., in the conical proventriculus with its broad base connected with the crop, the elongation and curvature of the ventriculus, which often completely encircles the intestine, and the division of the anterior intestine into at least two sections, the ileum being separated from the colon by a spiny zone, considered by Imms to be a valve ("pyloric valve"), but termed by Sutherland the "posterior gizzard" and believed by her to "grind any larger particles of food which have been left over by the anterior gizzard before they enter the large intestine" (colon).

Holmgren points out that in primitive termites such as *Hodotermes* the crop is a large sac, as in the *Blattodea*, but in most of the genera it is reduced and restricted to the abdomen, while the oesophagus is greatly elongated and the proventriculus tends to be larger than in primitive genera. A specialized feature of the proventriculus of most Isoptera is a more or less pronounced neck or *collum* between the bulb and the ventriculus. Judd (33), who describes the proventriculus of *Termopsis*, states that "its inner lining resembles more closely that of the Blattodea than of the Mantodea as it has longitudinal folds rather than anastomosing ridges between the teeth".

In the numerous genera described by Holmgren there are no true gastric caeca, but in the primitive genus *Archotermopsis* there are five short diverticula at the anterior end of the stomach (Imms, 31). Sutherland, who investigated the anatomy and histology of a number of Australian termites, including the very primitive *Mastotermes*, makes no mention of gastric caeca in that genus.

In all these structures and also in the intima of the proventriculus, *Grylloblatta* differs from both Isoptera and Blattodea. The proventriculus is narrowed into a neck in front of the bulb but not behind, the ventriculus is short and wide and there are only two incipient gastric caeca which are broadly rounded. The intestine is much shorter than in the Isoptera and the anterior part is simple. The malpighian vessels are more numerous than in the Isoptera, in which there are not more than eight and frequently only four or two. Even when only four are present they are grouped into two pairs, while in *Grylloblatta* they are about equally spaced.

PHASMIDA

The digestive organs in this order have been described by Bordas (7, 11), who investigated various genera, and by Heymons (27) and Cameron (13), who made more detailed studies of *Bacillus rossii* Fabr.

The alimentary canal in this order differs considerably from those of other orthopteroid insects in a combination of features, some of which are unique. Its most striking peculiarities are the following:—

(1) The straight course of the entire tube.

(2) The relative short and narrow crop.

(3) The thin-walled and ill-defined proventriculus.

(4) The elongation of the mid-gut and its division into two specialized regions, the anterior characterized by well developed circular muscles arranged in annular bundles and associated with a pronounced transverse folding of the walls, while the posterior region is thinner-walled and is characterized by the presence of tubular filiform organs.

(5) The absence or rudimentary development of gastric caeca.

(6) The numerous malpighian vessels grouped into a number of small bundles, each opening into the summit of a small conical papilla.

In the lack, or feeble development, of gastric caeca, the Phasmida resemble the Dermaptera, while in the straight course of the digestive tube and the absence of a definite thick-walled proventriculus they are like the Plecoptera and the suborder Caelifera of the Orthoptera.

SALTATORIA

The order Saltatoria consists of two very distinct suborders, the *Caelifera* and the *Ensifera*. The digestive system in these two suborders is so different as to suggest that the Saltatoria may not be an entirely natural order (*vide* Discussion). It will, therefore, be convenient to consider these two groups separately.

The suborder Caelifera includes two superfamilies *Acridoidea* and *Tridactyloidea*, the close affinity of which has only comparatively recently been thoroughly cleared up by the work of Carpentier (16, 17) and Ander (1, 2).

Of the four families of Acridoidea, the Acrididae comprise the great majority of all the Caelifera. In this family the digestive systems of many species, representing five of the subfamilies, have been described by Bordas (10, 11), and more recently a number of additional forms have been brought to light by several workers, e.g., Tietz (51) and Hodge (28, 29). The range of variation among these forms is remarkably slight and the differences from our point of view are negligible.

The salivary glands in the Acrididae tend to be reduced and diffuse, and the reservoirs are inconstant, being sometimes absent. The alimentary canal is straight or nearly so, the oesophagus is rather short and the crop well developed but not very voluminous. The proventriculus is not externally apparent, being tubular without thickening of the muscular coat and with very little specialization of the epithelial and cuticular lining. The ventriculus is straight, cylindrical, and usually rather short and wide. There are six conical gastric caeca, directed forward, each with a posterior diverticulum applied to the surface of the ventriculus.

The anterior intestine is sometimes straight and of nearly uniform caliber, but it is more often divided into a broader anterior part, which is straight, and a constricted posterior part, which is often bent downwards in the sagittal plane. The malpighian vessels are long and numerous, numbering commonly 100 to 120, although sometimes only 30 to 40 (Bordas, 11). They are generally more or less distinctly grouped into fascicles, some of which are directed forward and others backward.

The alimentary canal of *Tetrix* (*Acrydium*), representing the family Tetrigidae, was first made known by Dufour (20) and has recently been described by Carpentier (16, 17). It differs from that of the Acrididae in the very short oesophagus and crop, the relatively much longer ventriculus, and the absence of the posterior diverticula from the six fingerlike gastric caeca. There are a dozen groups of malpighian vessels. The presence of two large salivary reservoirs also differentiates *Tetrix* from most of the Acrididae.

The other two families, Proscopidae and Pneumoridae, have not been studied.

The digestive organs of the Tridactylidae were first described briefly by Dufour (20), who studied *Tridactylus variegatus*. He noted their similarity to those of *Tetrix* and was the first to cast doubt upon the accepted classification of this genus among the Gryllidae. Nearly a century later Carpentier

(16) described and figured the gross features of the digestive organs of *T. thoracicus* and demonstrated the similar plan of structure of these organs in the Tridactylidae, Cylindrachetidae, and Tetrigidae.

The oesophagus and crop are even shorter in *Tridactylus* then in *Tetrix*, the crop being also very little inflated. The ventriculus, like that of *Tetrix*, is the longest section of the digestive tube and the largest in caliber. In *T. thoracicus* it is produced cephalad into four conical gastric caeca (Carpentier, 16), in *T. variegatus* (Dufour, 20; Fernando, 24) there are three, two larger dorsolateral and one smaller midventral, while Judd (33) found only the two lateral ones in *T. apicialis*. This number was also noted by Ander in the tridactylid genus *Rhipipteryx*. The intestine is straight and its divisions are similar to those usually found in the Acridoidea. Fernando describes the 32 malpighian vessels in *T. variegatus* as being arranged in a simple circle, not in tufts as described by Carpentier for *T. thoracicus*. He also differs from Dufour in his statement that the salivary glands are without reservoirs, since these are distinctly shown in Dufour's figure of the same species.

Cylindroryctes, of the family Cylindrachetidae, resembles *Tetrix* in having six slender gastric caeca, directed forward and having no posterior diverticula (Carpentier, 16). It differs from all the other groups in the extremely long and slender oesophagus and comparatively long, although little inflated, crop. This difference, however, is,what would be expected from the greatly elongated thorax, associated with the habit of burrowing into plant tissues. The ventriculus measures more than half of the length of the abdomen and, as in *Tetrix* and *Tridactylus*, is the largest in caliber of any part of the alimentary canal. The intestine is only about half as long and appears in Carpentier's figure to be about equally divided into anterior intestine and rectum. There are numerous malpighian tubules in many groups, but the exact number is unknown.

The common characteristics of the alimentary canal in the Caelifera may thus be summarized as follows: The salivary glands tend to be diffuse. The alimentary canal is straight or nearly so. The crop is not voluminous, sometimes very small, and the proventriculus is "comparatively simple in structure, being tubular in shape and having no heavy armature in the interior" (Judd, 33). The ventriculus is straight and of large caliber and the gastric caeca, which are typically six but may be reduced to 4, 3, or 2, are always slender and conical. The anterior intestine is short and straight, or only slightly bent, and is usually constricted just before the rectum. The malpighian vessels are numerous and generally grouped into six or 12 tufts.

The suborder Ensifera comprises the two superfamilies Tettigonioidea and Grylloidea. Six families have been recognized in the Tettigonioidea by recent workers and two in the Grylloidea.

Of the six tettigonioid families, by far the largest and most varied is the Tettigoniidae. In this family the digestive organs of a large series of forms were studied by Bordas (11), the proventriculus in particular by Graber (25), Ramme (41, 42), Wilde (58), and Judd (33), while the features that are important from the comparative standpoint of this and other families of Ensifera have been described by Ander (2).

The alimentary canal in the Tettigoniidae is long and convoluted. The oesophagus is usually short but the crop is large, often extending into the abdomen, and usually widest near the posterior end. According to Ander (2) it is much more inflated dorsally than ventrally, and this is characteristic of the Tettigonioidea in general. The proventriculus is a muscular bulb connected with the crop by a very short collum, which is merely a connecting passage with sphincter function. The intima is elaborated into a complex armature consisting of (*a*) six longitudinal columns of sclerites, each including a median and two lateral teeth, and flanked by a series of "barbated lobes", and (*b*) an alternating series of six longitudinal sclerotized ridges. The proventriculus is constricted again at its junction with the ventriculus, into which it opens by the stomodaeal valve. The valve is generally prolonged into two large free flaps, dorsal and ventral, projecting freely into the ventricular cavity. The proventriculus is embraced, and often largely concealed, by the two broadly rounded gastric caeca. These are slightly displaced, the dorsal caecum leaning to the right (Ander, 2). The lining epithelium is folded into parallel lamellae of varying height, which serve to increase the secretory surface and divide the cavity of the caecum, partially or (rarely) completely, into a number of chambers. In certain Pseudophyllinae and Mecopodinae (Bordas, 11; Karny, 34) each caecum has a slender posterior diverticulum, somewhat reminiscent of those found in the Acrididae, but undoubtedly this is a specialized feature (Ander, 2).

The ventriculus is a long tube, sometimes twice as long as the body, and more or less convoluted, sometimes forming a single loop, sometimes three or four. It is not divided into specialized regions and is either uniform in caliber or somewhat broader in front, where the caeca open into it. The intestine is generally shorter but the anterior part is sinuous or somewhat convoluted, although not further subdivided. The rectal sac is variable in size but is an ovoid chamber with six strong bundles of longitudinal muscles and six rectal glands, radially arranged at equal distances. The malpighian vessels, generally numerous (100 to 120), are typically grouped in six clusters, each cluster discharging into the intestine through one of the six ampullae. These may be evenly spaced or grouped in three pairs. Sometimes the number of ampullae is reduced to three or four, or the vessels themselves are not definitely grouped but irregularly arranged.

The salivary glands in the Tettigoniidae are generally voluminous, often covering the whole ventral surface of the thoracic floor and encroaching upon the sides and dorsal surface of the crop. They are usually divided into two sections, a smaller one in front and a larger behind. In *Salomona* (Bordas, 11) the salivary glands are compact and lie in contact with one another on the dorsal surface of the oesophagus and base of the crop, a position recalling that of *Grylloblatta*. Salivary reservoirs are usually present but are not seen in Bordas' figure of *Salomona*.

The digestive system of the Stenopelmatidae has been studied in *Hemideina* (Maskell, 36) and in *Stenopelmatus* (Davis, 18). The salivary glands are not very voluminous in either genus, although the reservoirs are well developed. The alimentary canal does not differ from that of the Tettigoniidae in any important respect. There is no definite collum between crop and proventriculus, the lining of which is very similar to that of the Tettigoniidae. The gastric caeca may or may not have low lamellae (absent in *Hemideina*) and the free flaps of the stomodaeal valve are as in the Tettigoniidae. The ventriculus is shorter than in the latter family, particularly in *Hemideina*, while the anterior intestine in this genus is much longer and has a short section of narrow caliber, termed by Maskell the "ileum", followed by a long expanded section, called the "colon", and the constriction just before the rectum is unusually long. In *Stenopelmatus*, however, the intestine is much shorter and quite like that of the Tettigoniidae. The widest part, termed "colon" by Maskell, is the "ileum" of Davis, who applies the term "colon" to the constricted region just in front of the rectum. The malpighian vessels are divided in the typical tettigoniid fashion in six equidistant groups, discharging through as many ampullae, into the intestine. There are about 200 tubules in *Hemideina* while in *Stenopelmatus* they were found to vary from 180 to over 300.

In the Rhaphidophoridae noteworthy features are —: the relatively short ventriculus, which is expanded anteriorly into a sort of vestibule for the broadly rounded gastric caeca, whose inner lining is devoid of lamellae; the presence of three equidistant free lobes of the stomodaeal valves, projecting into the caeca; and the reduction of the ampullae of the malpighian vessels to four, three, or only two. The writer found only two in *Ceuthophilus pallidipes*, close together on the inner curvature of the intestine.

In the Gryllacrididae the crop, according to Ander (2), is large, bulging posteriorly, and is sharply defined from the rather long proventricular collum. Bordas (11) depicts these features in his figure of "*Gryllacris aurantiaca*". Karny (34), however, describes the crop of *G. signifera* as more "primitive" and less like a caecum than in the Gryllidae and in his figures it appears decidedly small and without any posterior bulging, tapering in fact to its junction with the collum.

The gastric caeca are large in the Gryllacrididae, while the ventriculus is decidedly short for a family of Ensifera. In Bordas' figure cited above, all the malpighian vessels empty into a single ampulla, and this character, together with the posteriorly inflated crop and the long collum joined to it asymmetrically, was believed by Bordas to indicate affinities between the Gryllacrididae and the Gryllidae. Ander, however, dissected a number of species of Gryllacrididae and found the single ampulla to be exceptional in this family, the majority of species observed having six, the usual number in the Tettigonioidea.

According to Ander the Schizodactylidae share with certain Gryllacrididae the posteriorly inflated crop and the comparatively long collum, and in both families the cavities of the gastric caeca are partially divided into chambers by two to seven lamellae, although in *Schizodactylus* the five chambers are almost complete. In this genus there are the usual six ampullae through which the malpighian vessels discharge into the pylorus.

One other family, the Prophalangopsidae, belongs to the Tettigonioidea, but it is represented with certainty by only the type genus *Prophalangopsis* with one species, *P. obscura* Walk., whose internal anatomy is unknown. The genus *Cyphoderris*, referred to this family by Zeuner (60), appears to be near the Stenopelmatidae and Tettigoniidae in the characters of the alimentary canal, including the minute structure of the proventriculus (Ander, 2).

The superfamily·Grylloidea is similar to the Tettigonioidea in the general structure of the digestive organs but there are a few special characteristics, which may be listed as follows:

(1) According to Ander (2) the crop is generally expanded ventrally instead of dorsally, and the stomodaeum is twisted from left to right, so that the expanded part of the crop lies on the left side and the gastric caeca may be swung into a more or less lateral position.

(2) There are four main lobes in the stomodaeal valve, all usually small and none in the primary sagittal plane.

(3) Differences in the armature of the proventriculus, which need not be specified here (*vide* Ander, 2; Judd, 35).

(4) The ventriculus is divided into two distinct regions, separated by a sphincter muscle, an anterior region, which is smooth both externally and internally, and a posterior region longer and of larger caliber, with an uneven outer surface bearing three rows of small evaginations, and an inner surface bearing short papilliform processes (Bordas, 12; Ander, 2).

(5) The malpighian vessels open into a single ampulla at the summit of a common duct or ureter, which discharges into the pylorus.

In the family Gryllidae the oesophagus passes gradually into the crop, the shape of which is not very different from that of the Tettigonioidea except in the usually ventral expansion and the more or less asymmetrical opening into the proventriculus which is somewhat in advance of the posterior end, on the right (ventral) side. There is a distinct although usually short collum proventriculi: the cavities of the gastric caeca are incompletely divided by low epithelial lamellae. The ventriculus is typically long and convoluted although short and wide in *Myrmecophila* (Schimmer, 46), which lacks the usual division into two regions. The intestine is shorter than the ventriculus and does not differ significantly from that of the Tettigonioidea.

The Gryllotalpidae, which have been studied by Bordas (11), Eberli (22), and Sayce (45), possess, in addition to the special grylloid features, a few of their own, viz:—

(1) The oesophagus is long, of uniform diameter, and opens abruptly into the crop.

(2) The expansion of the crop is mainly perpendicular to the general axis of the digestive tube, so that the organ appears like a caecum.

(3) The crop narrows again abruptly into a tube which is continued into the well marked collum proventriculi.

(4) A pair of "arborescent glands" open into the ventriculus close behind the openings of the two caeca.

(5) The peculiar structure of the posterior region of the ventriculus is continued into the anterior intestine.

The common characters of the Ensifera, excluding highly specialized features, may be summarized as follows:—

Salivary glands voluminous with large reservoirs: oesophagus gradually enlarging into an ample crop, which extends usually through the thorax, often into the abdomen, and reaches its greatest diameter near the posterior end. Proventriculus a muscular ovoid chamber, often with a short neck or "collum" connecting it with the crop, and with a highly developed cuticular armature arranged on a plan of six longitudinal columns of dentiferous sclerites, alternating with sclerotized ridges, the general plan subject to innumerable variations of taxonomic value. The proventriculus is constricted again at its junction with the ventriculus, into which project a small number of free flaps from the stomodaeal valve, the number and arrangement of which is more or less characteristic of the various families and subfamilies. Proventriculus embraced by the two broad, rounded gastric caeca, which are primarily dorsal and ventral, but are generally more or less twisted from left to right so that they assume an oblique or even transverse position; their cavities usually incompletely divided into a small series of chambers by parallel lamellae, formed by folding of the lining epithelium. Ventriculus generally a long and more or less convoluted tube; malpighian vessels commonly divided into tufts, each opening into one of the six, or fewer, ampullae, which open into the pylorus: intestine shorter than ventriculus, but not straight, the rectum ovoid and often large.

From the foregoing descriptions of the digestive organs of the two suborders of Saltatoria, Caelifera and Ensifera, we may make the following comparisons with *Grylloblatta*.

(1) In the Caelifera the salivary glands are usually diffuse and sometimes scanty; in the Ensifera they are generally voluminous, each set divided into two groups of glands. In *Grylloblatta* the glandular masses are more compact than in either suborder of Saltatoria and embrace the oesophagus more closely. The absence of salivary reservoirs has no comparative significance.

(2) The crop is usually small to moderate in size in the Caelifera, voluminous often extending well into the abdomen, and widest near the posterior end in the Ensifera and in *Grylloblatta*.

(3) The proventriculus in the Caelifera is tubular, indistinguishable externally, without thickened muscular walls and with very little specialization of the lining epithelium and cuticle. In both Ensifera and *Grylloblatta* it is ovoid with thick muscular walls, sometimes connected with the crop by a "collum" which is best developed in *Grylloblatta*. In the Ensifera it is lined by a specialized armature of dentiferous sclerites arranged in six longitudinal series, alternating with ridges, while in *Grylloblatta* there are 12 rows of backwardly directed lamellae, which are not firmly sclerotized.

(4) The gastric caeca in the Caelifera are slender and conical; there are primarily six but they may be reduced in number, sometimes to two, which are lateral. In the Ensifera and *Grylloblatta* there are two broadly rounded caeca, embracing the posterior part of the proventriculus. In the Ensifera they are said to lie in the primary sagittal plane: in *Grylloblatta* they appear to be lateral and ventrally confluent.

(5) The ventriculus is straight in the Caelifera, of large caliber, variable in length; in the Ensifera generally much longer, often much exceeding the body length, more slender, and convoluted or, when relatively short, at least curved; in *Grylloblatta*, very short but forming part of a convolution; widest in front, as in many Ensifera.

(6) Malpighian vessels vary in the Caelifera from about 30 to at least 120, in the Ensifera from 100 to 300, and in both groups they are typically divided into six fascicles, each discharging into the intestine through an ampulla. In a few cases, when the vessels are not numerous, e.g. *Tridactylus variegatus* (Caelifera) they open individually into the intestine. This is also the case in *Grylloblatta* in which there are less than 30 vessels.

(7) The intestine in the Caelifera is short and straight or nearly so; in the Ensifera longer and more or less looped or convoluted; of moderate length in *Grylloblatta*, the rectum being large and the anterior intestine very short and twisted similarly to that of some of the more primitive Ensifera; musculature feebler than in the Saltatoria.

It is evident from the above comparisons that *Grylloblatta* has much more affinity with the Ensifera than the Caelifera, in respect of the digestive organs, and closer affinity with this group than with any other group of orthopteroid insects that we have considered. In fact the external appearance of these organs compares closely with those of the Ensifera, the chief differences being the more compact salivary glands, the long *collum proventriculi* and the short ventriculus. If the gastric caeca are truly lateral and those of the Ensifera actually dorsal and ventral, then the case for affinity between *Grylloblatta* and the Ensifera is certainly weakened. Otherwise the greatest difference lies in the specialization of the inner lining of the proventriculus, which in the Ensifera is generally considered to function as a triturating organ, while in *Grylloblatta* this is obviously not the case.

The question as to which family of Ensifera most nearly approaches *Grylloblatta* is of little moment, since the common ancestor of the Ensifera and Grylloblattaria must have been a form that existed before any of the modern families had yet evolved. This is indicated by the numerous primitive characters still possessed by *Grylloblatta*, such as the cursorial legs, the five-jointed tarsi, the segmented cerci, movable coxites of the male, presence of a metathoracic spinasternite, etc.

As far as the digestive organs are concerned there is little to choose among the families of Ensifera. The short mid-gut and anterior intestine of *Grylloblatta*, perhaps correlated partly with a largely carnivorous diet and partly with the extremely low metabolic rate, is most nearly approached by the Rhaphidophoridae, which resemble *Grylloblatta* in the relatively short ventriculus and anterior intestine and the short, broadly rounded gastric caeca, in which there are no epithelial lamellae. *Grylloblatta* is also somewhat grylloid in the form of the crop, in that the inflation appears to be chiefly ventral and the posterior end is somewhat bulging, and in the long proventricular collum whose junction with the crop is slightly asymmetrical. Some of the Gryllacrididae appear also to share these features with *Grylloblatta.*

The compact salivary glands of *Grylloblatta*, with their lack of reservoirs, and the simple arrangement of the malpighian vessels, are approached to some extent by certain Ensifera, notably *Salomona megacephala* (Bordas, 11), but such features are evidently secondary, as the more scattered form of salivary glands with their well developed reservoirs are general throughout the orthopteroid orders, and the division of the malpighian vessels into tufts, typically six, each discharging into the intestine through an ampulla, is also a general orthopteroid feature.

Discussion

Affinities of Grylloblatta

Comparison of the digestive organs of *Grylloblatta* with those of other orthopteroid groups reveals definite indications of its affinity with the saltatorian suborder Ensifera and with this group only. This is in conformity with our findings in the other papers of this series (Walker, 53-56). *Grylloblatta* differs considerably, however, from any known family of Ensifera in the inner structure of the proventriculus, as well as in many other features referred to in the previous parts of this series. We believe, therefore, that the Grylloblattidae can scarcely be classified as merely a very generalized family of Ensifera, as done by Handlirsch (*vide* Ander, 12), but is worthy of at least subordinal rank. The question then arises as to whether the nearest relatives of the Ensifera are the Caelifera or the Grylloblattaria.

Zeuner (60) claims that the ancestral line of the Ensifera may be traced back to "primitive stenopelmatoid" forms, which are, in part, derived from the family Oedischiidae of the Upper Carboniferous, a protorthopteran family, which possessed jumping hind legs. From the primitive stenopelmatoid forms arose another line which gave rise to the Stenopelmatinae, Gryllacridinae, and also, through the Jurassic family Locustopsidae, to the Caelifera. Thus Zeuner attempts to trace all recent saltatorial groups to saltatorial protorthopteran ancestors (Protosaltatoria).

Ander (2), from the evidence of extensive comparative studies of all the organ systems as well as the skeletal features and musculature, concludes that the Caelifera cannot be derived from the Ensifera and that these two suborders must have evolved independently from different protorthopteran families. He agrees with Zeuner in tracing both lines of descent back at least to the Upper Carboniferous, but differs greatly in the details of their evolutionary history. Such details, however, scarcely affect the systematic position of *Grylloblatta*. The significant point in Ander's conclusions is that he represents the basal stems of the two lines of descent as parallel lines; he does not attempt to trace them to a common ancestor.

Bearing Ander's conclusions in mind, and in consideration of the many features that link *Grylloblatta* with the Ensifera, we venture to suggest that the common ancestor of the Ensifera and Caelifera is to be sought, not in the Protosaltatoria, but in some earlier family of cursorial Protorthoptera, and that the saltatory habit found in both Ensifera and Caelifera must have been independently acquired during the evolution of these two groups, although possibly through the recurrence of the same mutation. *Grylloblatta* would thus be derived from the Ensiferan stock before the saltatory habit had arisen. The Ensifera and Caelifera are thus parallel groups, not only in the possession of saltatory hind legs but also in the reduced number of tarsal joints, and the unsegmented cerci. Evidence that primitive members of both suborders possessed segmented cerci is found in the genus *Speleiacris* of the Ensifera (Ander, 2) and the genera *Tridactylus* and *Rhipipteryx* of the Caelifera, all of which have two-jointed cerci.

This view of the relationship of *Grylloblatta* to the Saltatoria and of the two suborders of the latter to one another differs from that which we have hitherto expressed, in that we were unwilling to admit the dual origin of the saltatory habit and the other parallelisms that are associated with it.

Function of the Proventriculus of Grylloblatta

The most characteristic features of the alimentary canal of *Grylloblatta* appear to be found in the proventriculus, of which the inner structure is strikingly different from that of all other orthopteroid groups and the collum is unusually long and muscular. The great development of the muscle layers of the proventriculus contrasts strongly with the feeble musculature of other parts of the alimentary canal, especially the rectum, whose muscle layers contrast with those of most mandibulate insects, in which they are thickened for the expulsion of faeces. Some consideration, therefore, of the function of the proventriculus seems to be warranted, even though it is merely deduced from the anatomical findings.

The problem of the function of the proventriculus of insects has been discussed at great length and the published work on the subject has been reviewed from time to time by various authors, such as Rungius (43), Ramme (41), Davis (18), and Deegener (19).

The earlier insect anatomists of the 19th century assumed from its structure that the proventriculus in mandibulate insects functioned as a "gizzard", i.e., an organ for further trituration of the food material after it had been acted upon by the jaws. The complex arrangement of hard toothlike structures lining the cavity of this organ in many insects, and the thick muscular walls, seemed to afford sufficient evidence for this manner of functioning. Such a view is assumed by Dufour (20) and by later authors, such as Graber (25), Wilde (58), and Bordas (11).

Wilde, however, having observed the atrophied condition of the proventriculus in the Acrididae, and the presence of toothlike structures in the anterior part of the crop with thickening of the muscles in this region, believed that in this group the function of the "gizzard", in so far as it existed, was taken over by this modified anterior part of the crop.

While a few workers before the middle of the century cast doubt upon the generally accepted view that the proventriculus of Orthoptera was a gizzard, it was Plateau (40) who exercised the greatest influence on the opinions of later writers. Plateau cites the view of Milne Edwards that the terminal part of the proventriculus was a valve for regulating the movement of food from the crop into the ventriculus, although the anterior part of the organ was a gizzard. Plateau himself claimed that the entire proventriculus functioned only as a regulatory valve and a filter for the food entering the ventriculus. Although his views were based primarily upon observations of adephagous Coleoptera, he extended them to include the Blattodea and the suborder Ensifera of the Orthoptera. He pointed out, e.g., that even in the tettigoniid *Locusta* (now *Phasgonura*) *viridissima*, the vegetable fibers of the food in the proventriculus were found lying in the grooves between the tooth-bearing ridges with their long axes parallel with that of the gut. Plateau denied any triturating function of the proventriculus in either Coleoptera or Orthoptera and, under his influence, this view was widely adopted by a majority of workers, including Petrunkewitsch (39), who experimented with the cockroaches *Blatta* and *Blattella*, and Krüger (35), who worked especially with a pselaphid beetle, *Claviger testaceus*, but applied Plateau's conclusions very generally. Rungius (43) criticized Krüger and others for their generalization that, because the proventriculus functions as a valve and sieve, and is sometimes so degenerate that it could not function as a gizzard, it could therefore never do so. He points out, too, the many outstanding authors who still favor the older view—Bordas (11), Deegener (19), Schimmer (46);

and, above all, Berlese (4). His own work on the proventriculus of *Dytiscus*, which is mainly anatomical, he considered to support the view that this organ is a true gizzard. He thought that this view could be confirmed by examining the crop of a *Dytiscus* just after feeding the insect with meat. The presence in the crop of large coarse pieces of meat just after feeding and the fact that the crop is empty after 24 hr., and the mid-gut contains a fine "brei" showed, in his opinion, that the meat had been ground up by the gizzard into fine particles before being passed on into the mid-gut.

Ramme (41, 42) who made feeding experiments with *Dytiscus*, several Carabidae, and various Orthoptera, agreed with Plateau that the proventriculus does not function as a second triturator of the food but he showed by many experiments that, in all the insects investigated, the gastric secretion is passed forward through the proventriculus into the crop and mixed here with the food, the crop being a more important digestive chamber than had been hitherto realized. Another significant discovery was that in the Dytiscidae and Carabidae the indigestible particles of the food, such as fragments of cuticle, were vomited and never pass through the proventriculus. Ramme's final results were in accord with Plateau's in that he ascribed to the proventriculus only the function of a valve which permits the emptying of the gastric secretion upon the food in the crop and allows the dissolved parts of the food to be strained through into the stomach.

These results, however, are based on the study of Coleoptera and are not necessarily applicable to orthopteroid insects. Eidmann (23) investigated the structure and function of the proventriculus of the cockroach *Periplaneta* (now *Blatta*) *orientalis* and found that the organ was divided into two parts, anterior and posterior. The anterior part contains six radially arranged powerful teeth and is able to comminute hard food particles such as sclerotized cuticle. After comminution the food does not pass immediately into the mid-gut but *re-enters* the crop after some time, in order to be acted upon again by the secretion present there. The posterior part of the proventriculus is chiefly a closing apparatus or valve.

The Ensifera have the most powerful and elaborate system of hard toothlike structures of any group of orthopteroid insects and it is here, if anywhere, that we would expect to find them used as a triturating organ. Du Porte (21) has shown that in *Gryllus* chitinous particles are found in the mid-gut and that they are distinctly smaller than those in the crop. Davis (18) found that a wax pencil introduced into the opening of the proventriculus of *Stenopelmatus*, through a hole in the crop, received an impression of the teeth of the proventriculus.

While the general structure of the alimentary canal of *Grylloblatta* is undoubtedly more like that of the Ensifera than any other group of insects, and even the proventriculus, with its neck and bulb, shares outwardly in this similarity, the internal structure is so different as to indicate some very marked difference in function. Thus, whereas the Ensifera are the group in which the proventriculus most probably functions as a gizzard or triturating organ, that of

Grylloblatta is entirely lacking in anything resembling teeth. Although carnivorous, *Grylloblatta* feeds on soft-bodied insect larvae or ant pupae, and when offered mealworms it devours only the soft parts, rejecting the cuticle. The absence of a molar surface on the mandibles is probably significant in this connection, as is also the absence of a peritrophic membrane, although this feature should not be over-emphasized as it has been observed in only one series of sections. Other features that may be significant in this connection are (1) the large crop and the small stomach, (2) the feeble muscular layers of the alimentary canal, except in the proventriculus, which is decidedly muscular, both in the long neck and the bulb.

The possibility that the proventriculus of *Grylloblatta* may function as a "gizzard" may be eliminated at the outset, owing to the soft nature of the food and the lack of anything resembling teeth in this organ. The lining of the bulb with its double circle of flaps certainly suggests a regulatory valve, but the muscular neck and walls of the bulb, together with the feeble muscle layers elsewhere in the alimentary tract, indicate that the proventriculus may be something more than a mere valve.

Thiel (50) has shown that in certain Coleoptera the proventriculus functions chiefly as a propulsive organ for the food, being the only part of the alimentary canal in which the muscular coat is sufficiently well developed to act in this way. The food material is already in a finely divided, or even emulsified, state. In many Adephaga there are valvular plates in front and behind the chamber of the proventriculus. By peristaltic contractions of the muscular wall of the proventriculus the anterior valve is opened and the chamber is filled with a portion of the crop contents. Next the anterior valve is closed and the posterior valve opened as the contents of the chamber are forced into the stomach. In some Coleoptera the anterior valve is absent, and in *Grylloblatta* the muscular neck would preclude the need of such a valve. It is suggested then that the proventriculus of *Grylloblatta* may function somewhat in the following way. By peristaltic contractions of the muscular collum food is drawn from the crop and passed on to the bulb. While the contracted circular muscles of the proventricular neck close its lumen, the food is expelled from the chamber of the bulb into the stomach. The system of projecting lamellae in the bulb serves to prevent any backflow of the food stream. Since the crop is an important digestive cavity in other orthopteroid insects, it is presumably so also in *Grylloblatta*, the smooth grooves between the bases of the lamellae being apparently fitted for the forward passage of digestive secretions from the stomach, even when the bulb is contracted.

Digestion in *Grylloblatta* is doubtless very slow, since in captivity, at a temperature little above 0° C., it feeds usually only about once in three months. Hence the very thin muscular coat of the intestine may be correlated with the slow movement of undigested food materials along the intestinal tract.

Summary

A. The organs of digestion of *Grylloblatta campodeiformis* Walk. were studied from dissections of adult insects and one series made from a male nymph. The main findings were as follows:

1. The labial or salivary glands are compact and appear throughout most of their length as a single mass surrounding the oesophagus. Salivary reservoirs are lacking.
2. The three main divisions of the alimentary canal are a very large fore-gut or stomodaeum, a very short mid-gut or mesenteron, and a moderately short hind-gut or proctodaeum.
3. The fore-gut is divided into pharynx, oesophagus, crop, and proventriculus.
4. The crop is thin-walled and capacious, not distinctly defined anteriorly but widening posteriorly until it reaches the third or fourth abdominal segment. The inflation is mainly ventral and somewhat sinistral, owing to a slight dextral twist, and the passage into the proventriculus is dorsodextral.
5. The proventriculus is very muscular and consists of a long neck or *collum* and a rounded bulb, terminating in a short stomodaeal valve.
6. The inner lining of the proventriculus is arranged on a plan of 12 instead of the usual six longitudinal divisions. These terminate behind in two alternating series of free, flexible processes, there being no hard, tooth-like structures.
7. The mid-gut consists of one short, wide chamber, the ventriculus or stomach, which is produced forward into a bilobed sac, embracing the bulb of the proventriculus and representing a pair of incipient gastric caeca.
8. There appears to be no peritrophic membrane.
9. The hind-gut is of moderate length and curved into a partial loop. It is divided into the anterior and posterior intestines.
10. The anterior intestine is uniform in caliber and histological structure throughout its length, there being no division into ileum and colon. It is short and forms with the mid-gut a partial loop.
11. The malpighian vessels are moderately numerous (12 to 24) and arranged in a simple annulus around the pylorus, each opening separately.
12. The posterior intestine or rectum is much wider than the anterior and is divided into a wide rectal sac and a narrower rectum proper. The rectal sac contains six rectal pads, arranged alternately.

B. A comparison was made between the gross features of the digestive organs of *Grylloblatta* and representatives of other orthopteroid orders, as described and figured in the literature. The orders represented were Dermaptera, Plecoptera, Blattodea, Mantodea, Isoptera, Phasmida, and Saltatoria (Suborders Caelifera and Ensifera).

C. The affinities of *Grylloblatta* are discussed on the basis of the above comparison of digestive organs with representative orthopteroid groups. The main conclusions are that:

1. *Grylloblatta* shows definite evidence of closer relationship to the suborder Ensifera of the order Saltatoria than with any other orthopteroid group.
2. While *Grylloblatta* resembles the Ensifera in most of the gross features of its digestive organs it differs strikingly in the inner structure of the proventriculus.
3. The differences in the proventriculus together with those already described in other parts of the body are sufficient to warrant its classification in a group apart from the Ensifera, and even from the Saltatoria.
4. The common ancestry of the Ensifera and Caelifera is more remote than that of the Ensifera and Grylloblattaria.

D. The probable function of the proventriculus of *Grylloblatta* is considered on the basis of its peculiar structure. Following a review of the more important papers on the function of the proventriculus in mandibulate insects, it is deduced from the structure of this organ in *Grylloblatta* that it probably functions not only as a regulatory valve but also as a propulsive organ, food being drawn from the crop by the muscular collum and passed into the bulb, whence by contraction of the muscular walls of the bulb it is delivered into the stomach. The very thin muscular layers of the intestine may be correlated with the extremely slow digestion of this insect, associated with the low temperatures at which it is active.

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Explanation of Figures

Plate I

Grylloblatta campodeiformis Walk., late female nymph, opened dorsally with head removed; general view of viscera, showing alimentary canal in natural position.

Fig. 1. *Right lateral view of a male nymph of Grylloblatta, showing the entire alimentary canal and salivary glands and their positions in relation to the regions of the body and the somites. Semidiagrammatic, based on a study of a series of sections, although no model was constructed. In the living insect the head is prognathous.*

Fig. 2. *Sagittal section of head with pharynx opened. 1, levator muscle of labrum; 6, 7, and 8, dorsal dilators of pharynx; 18, adductor of mandible; 23, adductor of stipes (these muscles are described in Part 1 of this series).*

Fig. 3. *Maxillae and labium with submentum and mentum removed to show the paired salivary ducts embracing the ventral nerve cord and continuing forward beneath the suboesophageal ganglion to the region of the mentun, where they unite to form the common or median duct.*

Fig. 4. *Transverse section through the prothorax, showing the labial or salivary glands, which appear as a single glandular mass overlying the oesophagus, with the prothoracic ganglion beneath it.*

Fig. 5. *Portion of a salivary gland to show the somewhat irregularly shaped acini, composed of large cells with deeply staining, basal nuclei and actively secreting cytoplasm.*

Fig. 6. *Portion of wall of oesophagus, showing the flattened and folded lining epithelium, covered with a thin cuticular intima; also the outer circular muscle fibers and inner longitudinal muscle fibers.*

FIG. 7. *Part of crop, proventriculus, ventriculus and pylorus, with proximal parts of malpighian vessels. Dorsal view.*

FIG. 8. *Entire intestine or proctodaeum, with malpighian vessels and rectal muscles. In the rectal sac the alternating arrangement of the rectal pads shows through the wall of the gut.*

FIG. 9. *Portion of wall of the bulb of the proventriculus, viewed from within, showing eight of the 12 anterior folds (a) and the alternating posterior series (b), which terminate in a row of wedge-shaped processes (c), the second series of processes or lamellae, in alignment with the anterior folds, being alternately longer (d) and shorter (e).*

FIG. 10. *Oblique section through junction of proventriculus and ventriculus, showing the lamellae arising from the epithelial lining of the former, with their cuticular intima, the stomodaeal invagination, and the wall of the ventriculus, the epithelium of which is in an active state of secretion and disintegration.*

FIG. 11. *A portion of the ventricular wall, showing groups of digestive epithelial cells in an active state of secretion, and basal regenerative cells, in a few of which may be seen mitotic figures.*

FIG. 12. *Section through the pyloric region of the wall of the alimentary canal, showing groups of ventricular epithelial cells, epithelium of the anterior intestine, malpighian vessels penetrating the wall of the pylorus and a part of the sphincter muscle (sph).*

FIG. 13. *Transverse section through the anterior intestine, showing folded epithelium and sections of malpighian vessels, the small sections cut near the bases, the larger ones farther distad.*

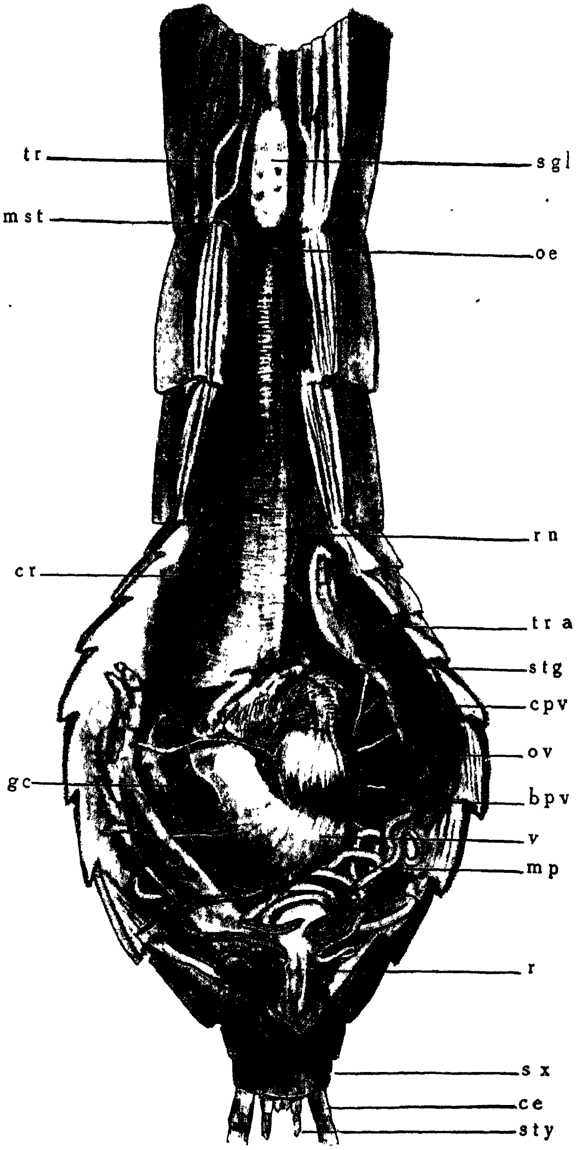
FIG. 14. *Transverse section through part of wall of rectal sac, showing a rectal pad and the extremely flattened epithelium lining the sac elsewhere.*

ABBREVIATIONS

bc —buccal cavity
bpv —bulb of proventriculus
c —cerebral ganglion
cb —cibarium
ce —cercus
cl —clypeus
clr —compressor of labrum
cpv —collum of proventriculus
cr —crop
cu —cuticular intima
dda —dorsal dilator of anus
ddr —dorsal dilator of rectum
ep —epithelium
epf —epithelial fold
ep in—epithelium of intestine
ep v —epithelium of ventriculus
gc —gastric caecum
gl —glossa
gms —mesothoracic ganglion
gmt —metathoracic ganglion
gpr —prothoracic ganglion
hy —hypopharynx
in —anterior intestine
li —labium
ln —labial nerve
lp —labial palp
lr —labrum
m —mouth
mc —circular muscle fibers
md —mandible
ml —longitudinal muscle fibers
mn —mentum

mp —malpighian vessels
ms —median salivary duct
mxn —maxillary nerve
mxp —maxillary palp
nc —nerve cord
oe —oesophagus
ov —ovary
ph —pharynx
pm —prementum
pr —preoral cavity
pv —proventriculus
r —rectum proper
rn —recurrent nerve
rp —rectal pad
rs —rectal sac
rtr —retractors of rectum
sd —paired salivary duct
sd 1 —branch of salivary duct
sgl —salivary gland
sm —submentum
sog —suboesophageal ganglion
sph —sphincter muscle
stg —stomodaeal ganglion
sti —stomodaeal invagination
sty —stylus
sv —salivarium
tn —tentorium
tr —trachea
tr a —trachea from first abdominal spiracle
v —ventriculus
vda —ventral dilator of anus
vdr —ventral dilator of rectum

(Plate I and Figs. 1–14 follow.)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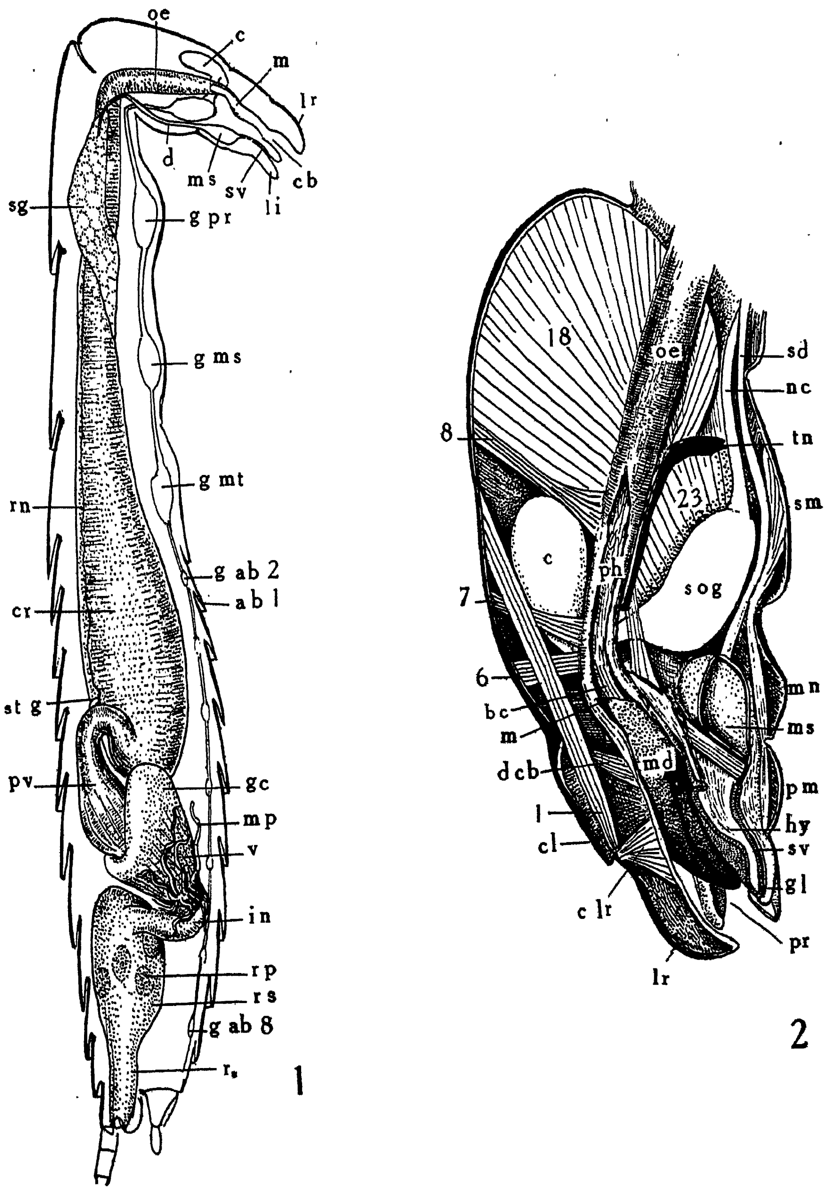
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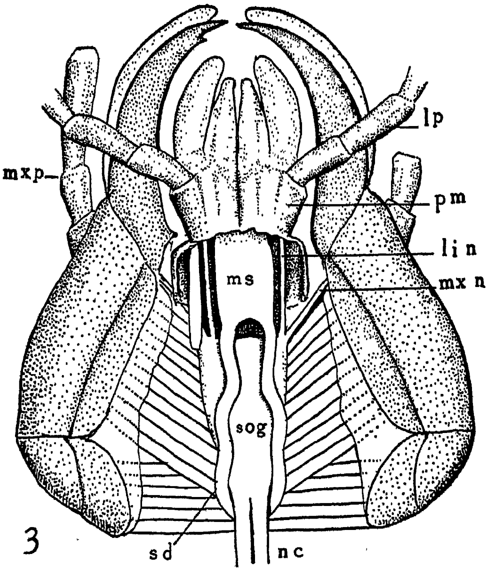
FIGS. 1 AND 2.



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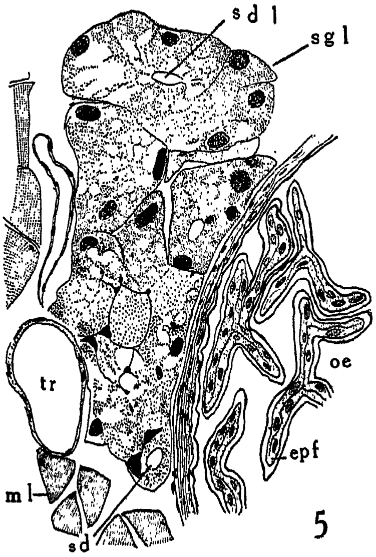

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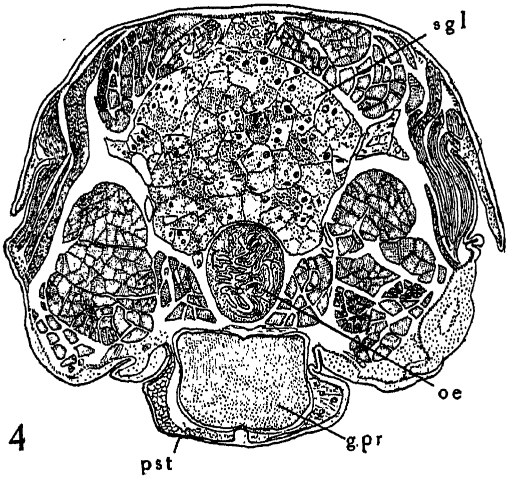

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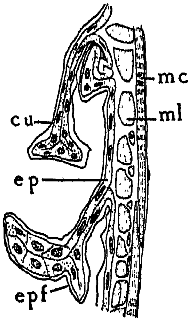

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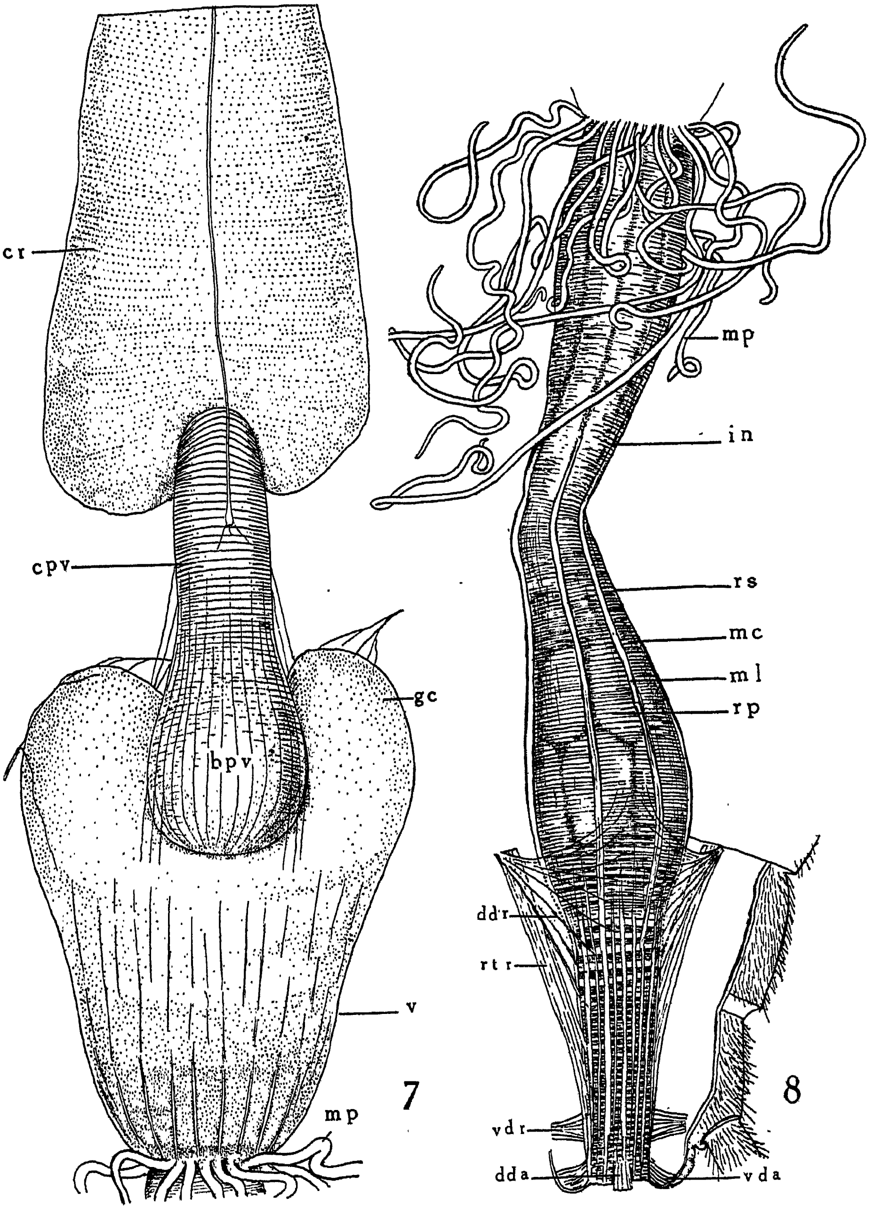
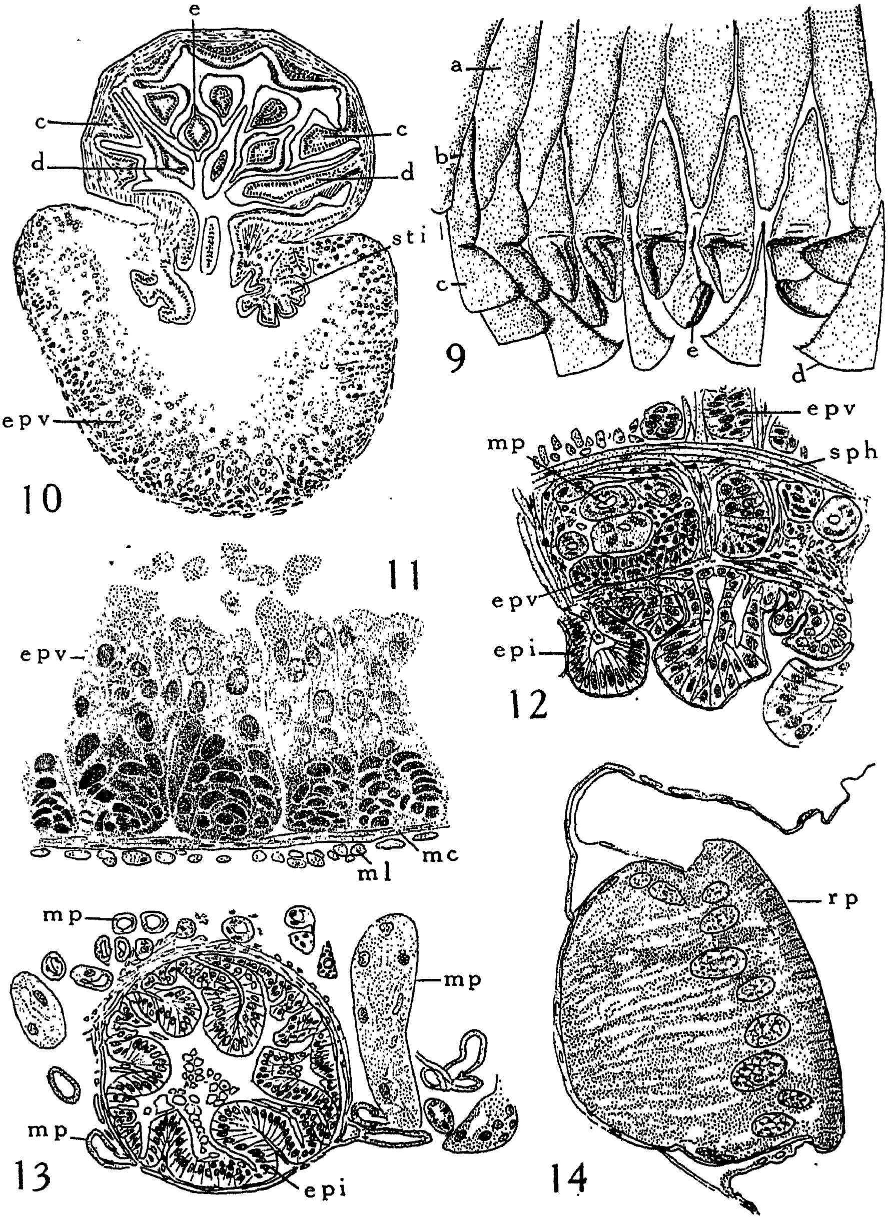
FIGS. 3 TO 6.



FIGS. 7 AND 8.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FIGS. 9 TO 14.

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